Advancements in Electrochemical and Optical Detection, Quantification, and Characterization of Cosmetic/Industrial Polyelectrolytes

by

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"... Keep working. Keep striving. Never give up. Fall down seven times, get up eight. Ease is a greater threat to progress than hardship. Ease is a greater threat to progress than hardship. So keep moving, keep growing, keep learning. See you at work."

-Denzel Washington

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DEDICATION

This work is dedicated to my family for their unwavering patience, love, and support. Without them, I would not be here today.

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ABSTRACT

Polyions are polymers that contain significant numbers of positive and/or negative charges dispersed along their polymeric chains. Polyquaternary ammonium salts (i.e., polyquaterniums (PQs)) are cationic polyions (polycations) that derive their positive charges from quaternary ammonium groups. These polycations have found increased use in various cosmetic and industrial applications (e.g., hair conditioning formulations, lotion formulations, water treatment processes, etc.).

As PQs are used in many applications it is imperative to develop simple devices/methods to quantitate and characterize these species. This dissertation examines the application of various electrochemical and optical sensing technologies to the study of four PQs (i.e., PQ-2, PQ-6, PQ-10, and poly(2-methacryloxyethyltrimethylammonium) chloride (PMETAC)). Chapter 1 provides an overview of the most significant advancements in electrochemical and optical polyion sensing technologies and applications over the last 25+ years. Chapter 2 explores the application of established electrochemical polyion sensing technology for the detection of PQ-10 in commercially relevant sample mixtures. These samples contain both sodium lauryl sulfate (SLS) and PQ-10. However, SLS can generate significant negative response signals when using single-use polyanion-sensitive ion-selective electrodes (ISEs) as detectors to detect PQ-10 indirectly *via* potentiometric titrations. However, it was determined that subjecting SLS/PQ-10 mixtures to an anion-exchange resin reduces the amount of SLS to a level at which there is negligible interference

observed. Further, the remaining PQ-10 is shown to be detectable within the range of 0 – 80 μg/mL by titration with dextran sulfate (DS).

Chapter 3 describes the application of single-use polyion-sensitive ISEs to the quantification and characterization of PQ-2 PQ-6, PQ-10, and PMETAC. Both direct (dose-response) and indirect (titrimetric) detection methods were explored. Limits of detection (3σ) for these species were found to be 7.7, 6.9, 21.5, and 9.0 μg/mL, respectively, using the preferred titration method. PQ-6 was also quantified in partially treated drinking water from a water treatment plant. Chapter 4 demonstrates the application of fully reversible (pulstrode) polyion-sensitive ISEs to the same PQ species discussed in Chapter 3 using direct and indirect detection methods. Limits of detection for PQ-2, PQ-6, PQ-10, and PMETAC were found to be 8.0, 7.9, 35.5, and 10.3 μg/mL, respectively, using the preferred titration method.

Chapter 5 explores the application of the first polymer-/plasticizer-free polycation-sensitive ion-selective optode (ISO) for the direct detection of PQ-2, PQ-6, PQ-10, and PMETAC. Each PQ was found to yield sigmoidal response curves when average hue values were plotted against the logarithm of the PQ concentration. These polycation-sensitive ISOs were found to be useful in quantifying PQ-6 in recreational swimming pool water. Chapter 6 describes the construction and application of the first all-solid-contact paper-based polyanion-sensitive ISE to the direct detection of polyanions and the indirect detection of PQs. These sensors are found to yield similar dose-response and titration data to the single-use ISEs described in Chapter 3. Slope values for each PQ calibration curve are in excellent agreement with calibration curves reported in Chapter 3. Finally, an overview of the accomplishments of this thesis work and potential new directions for polyion sensor research/applications are discussed in Chapter 7.

CHAPTER 1

Advances in Electrochemical and Optical Polyion Sensing: A Review

1.1 Introduction

The existence of multiply charged macromolecular species (polyions) provides a diverse and complicated field of study in a number of scientific disciplines, including but not limited to biology, chemistry, medicine, material science, macromolecular engineering, water treatment, molecular biology, and cosmetic chemistry. Interest in detecting polyions has increased significantly owing to their ability to package and pass on genetic information (i.e., DNA), treat patients to prevent medical complications such as deep vein thrombosis (using heparin), and aid Fortune 500 companies in developing effective personal care products (e.g., using polyquaternary species in soaps, detergents, shampoos, etc.). Hence, the number of fundamental and applied studies of polyionic species has soared over the last several decades.

Using the most simplistic chemical definition, polyions (also known as polyelectrolytes) are macromolecular species that contain multiple charges (i.e., positive and/or negative) along the

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length of their polymer chains. Polycations possess predominately positive charges (e.g., protamine and polybrene) while polyanions have primarily, or exclusively, negative charges along their chain lengths (e.g., DNA, heparin, dextran sulfate (DS), carrageenans, etc.) (see Figure 1.1 for examples of chemical structures). Some of the earliest scientific studies related to polyion measurements focused on what is arguably the most biomedically useful polyanionic species still widely used today in clinical procedures/surgeries: heparin.

One of the most significant debates in the early years of using heparin in patients was determining the best mode of heparin delivery to prevent thrombosis (i.e., continuously or intermittently). This debate was explored in depth by Gordon Murray in 1940;[1] continuous, intravenous infusion was the preferred method. Over the progression of the 20th century various methods have been developed for monitoring heparin levels in blood to ensure no excess heparin (risk of bleeding) or insufficient heparin (risk of clot/thrombosis) is present in the blood of patients undergoing clinical/surgical procedures. These methods include the Lee-White clotting time,[2] activated coagulation time (ACT),[3,4] partial thromboplastin time (PTT),[5] and the activated partial thromboplastin time (aPTT).[6] While these methods are well established, they depend on visible coagulation times, are not sensitive, are not specific to heparin (i.e., rely on the interaction of a variety of clotting factors), etc.[3,4,7] Given these limitations, more specific and reliable methods to quantitate heparin levels in blood are needed. To this end, the first reports of the application of traditional ion-exchanger-based polymeric membrane ion-selective electrodes (ISEs) to the detection/quantification of heparin in whole blood were introduced in the early 1990s.[8–10] This initial work led to significant research aimed at developing both electrochemical and optical chemical sensors to detect heparin as well as a wide range of other polyionic species in real-world samples.

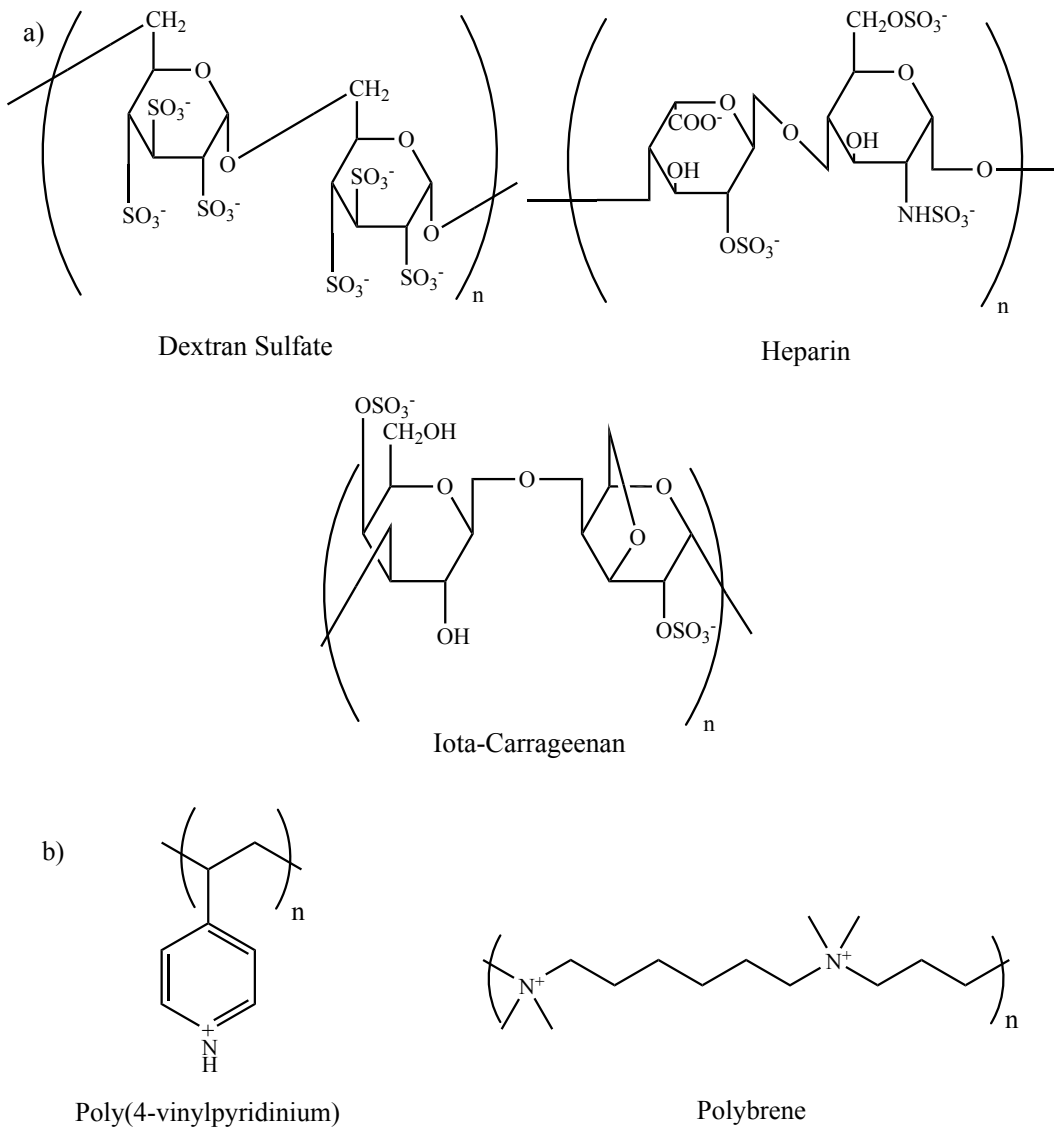

Figure 1.1. Chemical structures of example (a) polyanions and (b) polycations.

There are various other analytical techniques capable of quantifying polyions such as titration using a polyionic titrant in conjunction with Toluidine Blue O dye,[11] fluorescence,[12] and tannic acid precipitation.[13,14] However, these methods suffer from reliance on visual endpoints, complicated synthetic pathways/fluorescence tagging protocols, and excessive wait times, respectively. Quantitative LC-MS protocols have proven useful for detecting polycations but depend on sample pretreatments (e.g., hydrolysis using trifluoroacetic acid).[15] Even

electrochemical detection of DNA has seen advancements in recent decades,[16,17] although translation of these technologies to polyions such as heparin might prove difficult as electroactive guanine residues are not present in heparin and peptide nucleic acid (PNA) probes may not exhibit high affinity for another polyion other than DNA. Electrochemical and optical polyion sensors have overcome many of these difficulties. This chapter will review efforts in these areas over the past 25+ years.

1.2 Design and Features of Single-Use Polyion-Sensitive ISEs

Since 1992 there have been significant developments in the fabrication and application of polyion-sensitive ISEs. Many of these advances reference the same basic principles/design of the first reported heparin-sensitive ISEs.[9] This first design employed a plasticized poly(vinyl chloride) (PVC) membrane that incorporated the lipophilic anion-exchanger tridodecylmethylammonium chloride (TDMAC). The PVC membrane was plasticized with dioctyl sebacate (DOS).[9] Individual sensing membranes were integrated into macroelectrode bodies and used to detect polyanionic heparin in citrated human blood.[8–10] Potentiometric signals from these heparin sensors were measured against an external double junction Ag/AgCl reference electrode (RE). These initial "heparin" sensors exhibited reasonable response times (2-5 min) and could detect heparin levels in the clinically relevant concentration range (e.g., 0.1-10 U/mL).[9] The units of heparin concentration here refer to United States Pharmacopoeia (USP) units/mL.[8] One heparin unit is defined as the amount of heparin required to half-clot 1 mL of sheep blood plasma held at 37 °C within one hour.[18] Heparin USP units also correspond to mass values as described by the supplier. For example, a heparin sodium salt preparation from porcine intestinal mucosa provided by Sigma-Aldrich can contain 189 USP units/mg (dry basis). Similar heparin-sensitive ISEs were further

optimized and characterized by Ma et al.[8] and Yang et al.[10] by experimenting with various ion-exchangers, polymer matrices, and plasticizers to determine the optimal membrane composition that would generate the largest potentiometric response signal to heparin in solution or in citrated human blood. The optimal membrane cocktail composition was 66 wt. % PVC, 32.5 wt. % DOS, and 1.5 wt. % TDMAC,[8,10] which allowed limits of detection for heparin to reach values within the range of 0.1 – 1.0 U/mL.[10] These heparin sensors were denoted as "single-use" devices, since the extraction of heparin into the membranes is essentially irreversible. This is a result of the very favorable cooperative ion-pairing reaction between the polyanionic heparin and the $TDMA^+$ species within the membrane phase.[8,19]

The optimal TDMAC-based membrane formulation was shown to be applicable for the detection of many other polyanions, including carrageenan,[20] pentosan polysulfate (PPS),[21] fucoidan,[22] and oversulfated chondroitin sulfate.[23,24] Figure 1.2 shows the components used for a simple polyanion detection experiment using polymeric membranes in macroelectrode bodies. In these devices, a polyanion (e.g., heparin) is injected into a sample solution and polyanions present

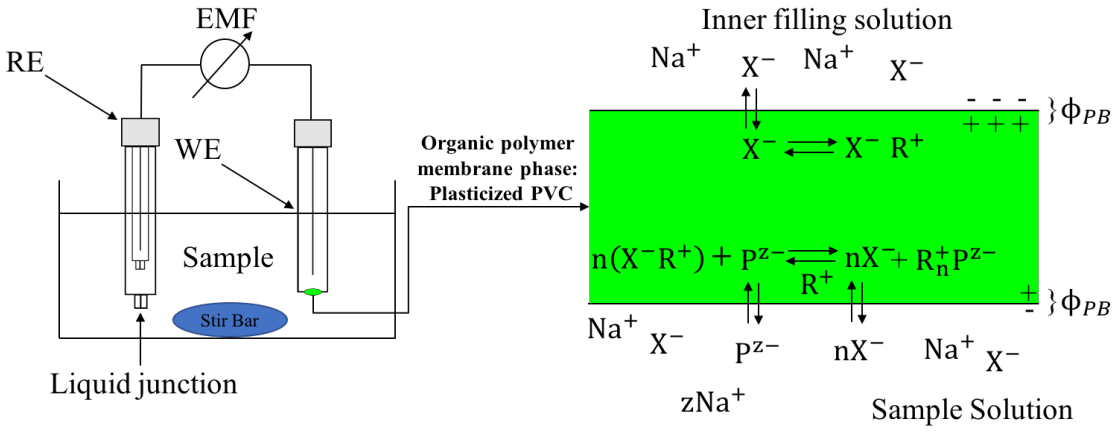

Figure 1.2. Setup of a polyion detection experiment using of a two-electrode system (working electrode (WE) and double junction reference electrode (RE)) placed within a sample contained in a beaker (left) and a diagram of the ion-exchange mechanism expected when a polyanion is extracted into the plasticized PVC membrane (right).

are extracted into the plasticized PVC membrane contained within the working electrode (WE) (see right side of Fig. 1.2). A high impedance voltmeter prevents substantial current flow between the WE and external double junction RE, and the change in the phase boundary potential between the sample and polymeric membrane phases is recorded.

It might be expected that these sensors would respond to circulating cell-free DNA in human blood. The concentration of such DNA in blood has been shown to be quite low for normal patients as opposed to patients suffering from various disease states (e.g., esophageal cancer, colorectal cancer, breast cancer, etc.).[25–28] These sensors do not show appreciable response to DNA at the low concentrations found in normal patients (i.e., exhibiting no disease state). However, if the concentration of circulating cell-free DNA in the blood increases enough in a patient suffering from one of these diseases, negative potential responses might be observed.

Interestingly, the EMF response of single-use sensors to polyanions occurs only over low and narrow concentration ranges. This behavior was ascribed to a non-equilibrium quasi steady-state potential model.[19,29] This model dictates that at low concentrations of a particular polyanion in the sample phase, the counterion to the ion-exchanger (e.g., Cl^-) within the plasticized polymeric membrane cannot be fully displaced over a short measurement period; the polyanion can only replace the counterions at the outermost layer of the plasticized polymer membrane.[19] The subsequent interaction of the polyanion with $TDMA^+$ forms cooperative ion-pairs due to the high charge density of the polyanion combined with the favorable thermodynamics resulting from the formation of a $TDMA^+$-based reverse-micelle scaffold around the extracted polyanion in the membrane phase.[20,29] These newly formed cooperative ion-pairs diffuse through the membrane bulk toward the inner filling solution.[30] This occurs at a much lower rate of diffusion within the polymeric membrane phase than that of the polyanion from the sample bulk to the sample-

membrane interface. The difference in the rates of diffusion allow the polyanion to accumulate within the membrane-sample interface, thus changing the interfacial potential.[19] Changes in the amount of plasticizer within the polymeric membrane phase can alter how fast the cooperative ion-pairs diffuse within the PVC membrane. For instance, decreasing the amount of plasticizer causes the cooperative ion-pairs to diffuse through the membrane bulk more slowly. This allows polyanions to accumulate within the polymeric membrane-sample interface more quickly than within a membrane with a higher plasticizer content.[19] This equates to lower limits of detection for membranes with lower plasticizer content. Limits of detection can also be improved by making use of rotating polyion-sensitive ISEs to increase the rate of mass transport of the polyions to the outer membrane surface.[31,32] It is important to note that once the polyanion has fully displaced the counterion to the ion-exchanger in the plasticized polymer membrane *via* excessive soaking times (even at low polyanion concentrations) or if the membrane is exposed to a high concentration of polyanion in the sample over a short time period, the expected equilibrium response predicted by the Nernst equation results (see Equation 1.1) (i.e., < 1 mV/decade for heparin, since the average charge on heparin is -70).[19] Equation 1.1 can also be applied to polycations.

$$E_{polyanion} = E^{0}_{polyanion} + \frac{RT}{z_{polyanion}F} \ln (a_{Polyanion}) \quad (1.1)$$

The electrochemical response of a polyion-sensitive ISE is typically a sigmoidal curve for E_{cell} vs. log(polyion concentration) plots. At the start of a simple polyion direct detection experiment, when there is no polyion in the sample phase consisting of an aqueous buffer solution containing a low concentration of NaCl, the measured potential is governed by the activity of the Na^+ or Cl^- in the sample phase following the Nernst equation for these singly charged species. As a polyion is injected into the sample phase the change in potential initially resulting from the

activity of the Na^+ or Cl^- equilibrium potential then follows a non-equilibrium response profile as the polyion is extracted into the polymeric membrane to form strong cooperative ion-pairs with the lipophilic ion-exchanger species (i.e., R^+ or R^-). The change in potential from the equilibrium potential in the presence of only the Na^+ or Cl^- activity is provided by Equation 1.2 and follows the sign of the analyte polyion where R is the universal gas constant, T is the temperature, F is the Faraday constant, z is the charge of the analyte polyion, $[R^{\pm}]$ is the concentration of lipophilic ion-exchanger in the membrane, D_a/D_m is the ratio of the diffusion coefficients for the polyion in the aqueous/membrane phases, δ_m/δ_a is the ratio of membrane/aqueous diffusion layer thicknesses, and $C_{polyion}$ is the concentration of the polyion in the sample phase:[19,30,33]

$$\Delta EMF = \pm \frac{RT}{F} \ln\left(1 - \frac{z}{[R^{\pm}]_{membrane}} \frac{D_a \delta_m C_{polyion}}{D_m \delta_a}\right) \quad (1.2)$$

Figures 1.3a-b show the general shape of the expected response curves for a polycation and a polyanion in simple direct detection experiments. These response curves are for polyions that have reasonable charge densities, molecular weights, diffusion coefficients in the polymeric membrane phase, etc. The curves further draw attention to the analytically relevant portion of each response curve in addition to the equilibrium response at higher polyion concentrations (i.e., "non-equilibrium response" and "equilibrium response," respectively). Figure 1.3c also shows a real heparin dose-response curve using a single-use polyanion-sensitive ISE. The non-equilibrium response modeled by Equation 1.2 of a polyion-sensitive ISE is only accurate for low levels of polyion within the sample phase. Once higher polyion concentrations are reached, the equilibrium potential toward the background of Na^+ or Cl^- written in terms of the activity of the analyte polyion will result and the ΔEMF of the response curve can be modeled using Equation 1.3 where $K_{exchange}$ is the ion-exchange constant of the polyion with Na^+ or Cl^- in the membrane phase:[33]

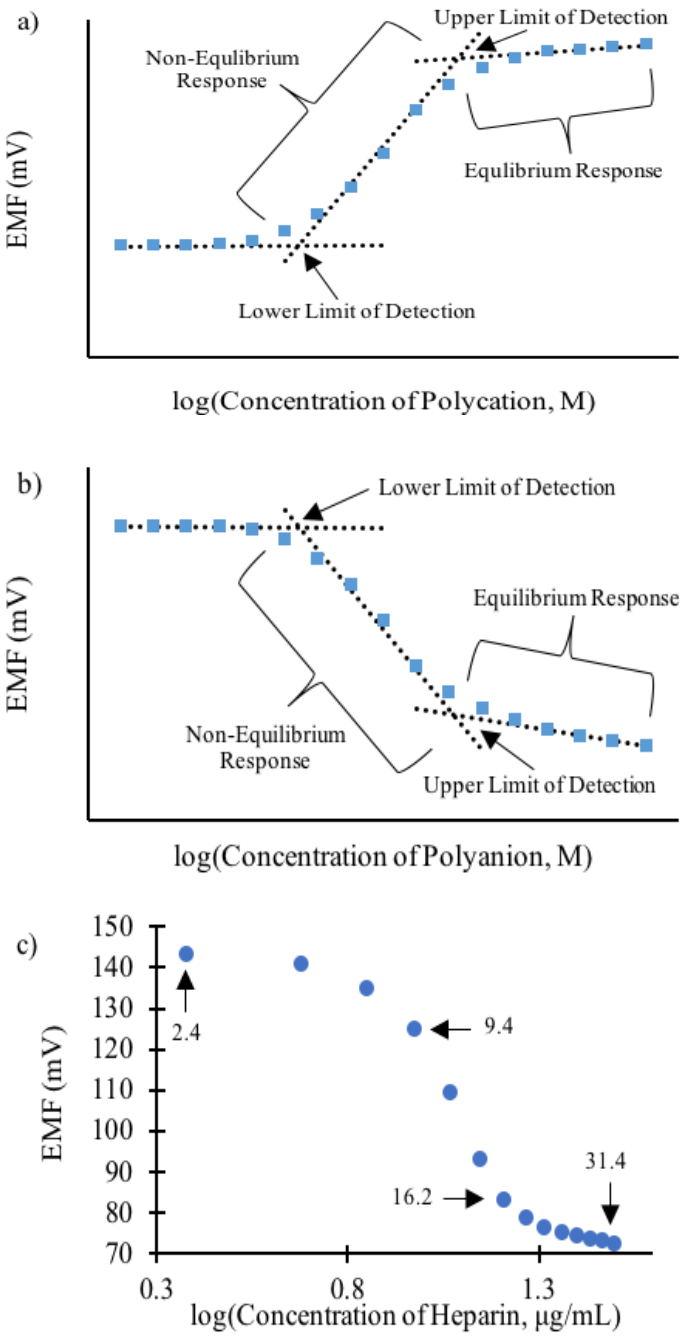

Figure 1.3. Simulated theoretical polyion response curves for (a) polycations and (b) polyanions. Analytically relevant non-equilibrium as well as equilibrium portions of each theoretical plot are indicated. The upper and lower detection limits are represented by the intersection of the extrapolated lines. Real heparin response potentiometric data is shown in (c) with indicated concentrations in µg/mL using a polymer membrane electrode containing 66 wt. % PVC, 32.5 wt. % DOS, and 1.5 wt. % TDMAC.

$$\Delta EMF = \frac{RT}{F}\ln\frac{a_{Na^+} \text{ or } a_{Cl^-}}{[R^{\pm}]_{membrane}} - \frac{RT}{zF}\ln\frac{zK_{exchange}C_{polyion}}{[R^{\pm}]_{membrane}} \quad (1.3)$$

The first detection of polycations with polymer membrane ISEs was reported by Yun et al.[34] using a polymer membrane containing potassium tetrakis(4-chlorophenyl)borate (KTpClPB), PVC, and 2-nitrophenyl octyl ether (NPOE) to detect protamine (the arginine-rich polypeptide that is used to reverse the anticoagulant activity of heparin) in solution. Han et al.[35] and Ramamurthy et al.[36] further explored the use of a different and inexpensive cation-exchanger, dinonylnaphthalenesulfonate ($DNNS^-$), in place of the previously employed KTpClPB, for preparation of protamine-sensitive ISEs to directly detect protamine and also to monitor serine protease activities (e.g., chymotrypsin and renin). Sensors employing the $DNNS^-$ cation-exchanger were shown to exhibit much higher total ΔEMF values (ca. 110 mV) toward the highest concentrations of protamine compared with membranes formulated with KTpClPB.[36] PVC membranes plasticized with NPOE and doped with $DNNS^-$ yielded the highest total ΔEMF responses to protamine in Tris-HCl, pH 7.4, with 120 mM NaCl because the polarity of NPOE is much higher than the polarity of the other plasticizers tested.[36] Polyurethane M48 membranes plasticized with NPOE and doped with $DNNS^-$ provided the lowest limits of detection toward protamine in undiluted human plasma because it exhibits more rigidity as compared to Tecoflex.[36] Therefore, the optimal membrane composition for protamine detection was suggested to be 49.5 wt. % polyurethane M48, 49.5 wt. % NPOE, and 1.1 wt. % $DNNS^-$.[36] Protamine-sensitive membranes formulated with $DNNS^-$ and PVC were later found to be useful as polyion sensors even when the sensing membrane is preconditioned in the analyte polyion[37,38] or when moving parts (i.e., stir bars) are removed from the polyion sensing experimental setup.[39] Polycation sensors prepared using PVC membranes containing $DNNS^-$ have also been demonstrated to be applicable

to sensing adenosine-5'-triphosphate (ATP) aptamers *via* protamine titrations in addition to indirectly quantifying ATP in a buffer solution.[40]

Different sensor permutations/configurations were explored in the early development of potentiometric single-use polyion sensors. A prototype solid-state heparin sensor was fabricated on a silicon substrate and exhibited a linear response to heparin when the absolute potential of the heparin-sensitive polymer film was plotted against the logarithm of the heparin concentration.[10] Also described were inexpensive/disposable heparin sensors for practical clinical use,[41] made by using coated wire-type electrodes containing the specially formulated polymeric membrane cocktail developed for heparin sensing.[8–10] Copper wires insulated with Tygon® tubing were employed as the electronic conductor onto which the membrane cocktail was dip-coated directly. These sensors were successful in the direct detection of heparin in citrated human plasma with a slope of -19.2 mV/dec and yielded similar mass values of protamine required to neutralize the heparin in human plasma as compared to human plasma samples containing identical concentrations of heparin and measured using the aPTT test.[41]

These early potentiometric polyion sensors established the basis by which electrochemical polyion sensing devices for the detection of other polyanions as well as polycations would continue to be fabricated and studied. The membrane-cocktail formulations used in subsequent studies have been remarkably similar to those of these initial devices. The low cost and ease of operation of these polyion sensors have made them ideal tools for a wide range of applications.

1.3 Fully Reversible Polyion-Sensitive ISEs

While single-use polyion sensors are reliable, robust, and facile devices, they cannot easily be reused unless soaked for extended time periods in high ionic strength solutions (e.g., ≥ 1 M

NaCl) to remove extracted polyions from the membrane phase.[8] In an effort to overcome this limitation, the first fully reversible polyion sensor was introduced by Shvarev and Bakker (see Fig. 1.4).[42,43] This technology employed a neutral lipophilic salt (tetradodecylammonium ($TDDA^+$) – $DNNS^-$) within a NPOE-plasticized PVC membrane. Membranes containing this salt do not exhibit any spontaneous potentiometric response toward small anions/cations or polyanions/polycations (i.e., no ion-exchange properties). Indeed, this new device deviated from conventional potentiometry-based devices by requiring the use of a three-electrode system (i.e., working, double-junction reference, and counter electrodes). Using this three-electrode system the membrane is first polarized by applying a short, electrical current pulse (e.g., ≤ 1 s) through an inner Ag/AgCl reference and an external Pt counter electrode (CE). This current serves to enrich the outer surface (sample side) of the membrane with either the lipophilic cationic or anionic species (depending on the direction of current). After polarization, the passage of current is stopped and the open circuit potential of the membrane electrode vs. the external RE in the presence of a given concentration of polyion is recorded for a short period (< 1 s). During this period the lipophilic ionic species that is moved toward the sample side acts as an ion-exchanger and extracts the target polyion into the membrane phase. The membrane is then re-equilibrated to its original state by applying a potential between the inner Ag/AgCl RE and external Pt CE which corresponds to the original open circuit potential of the membrane electrode vs. the external RE for 10-20 s. This final electrical pulse regenerates the membrane to its original state by ejecting the extracted polyions back into the sample phase. In summary, the overall process for the series of electrical pulses is as follows: galvanostatic pulse, zero-current, and potentiostatic pulse (see Fig. 1.4). The recorded measurement of the membrane potential value is only taken during the subsequent zero-current pulse.

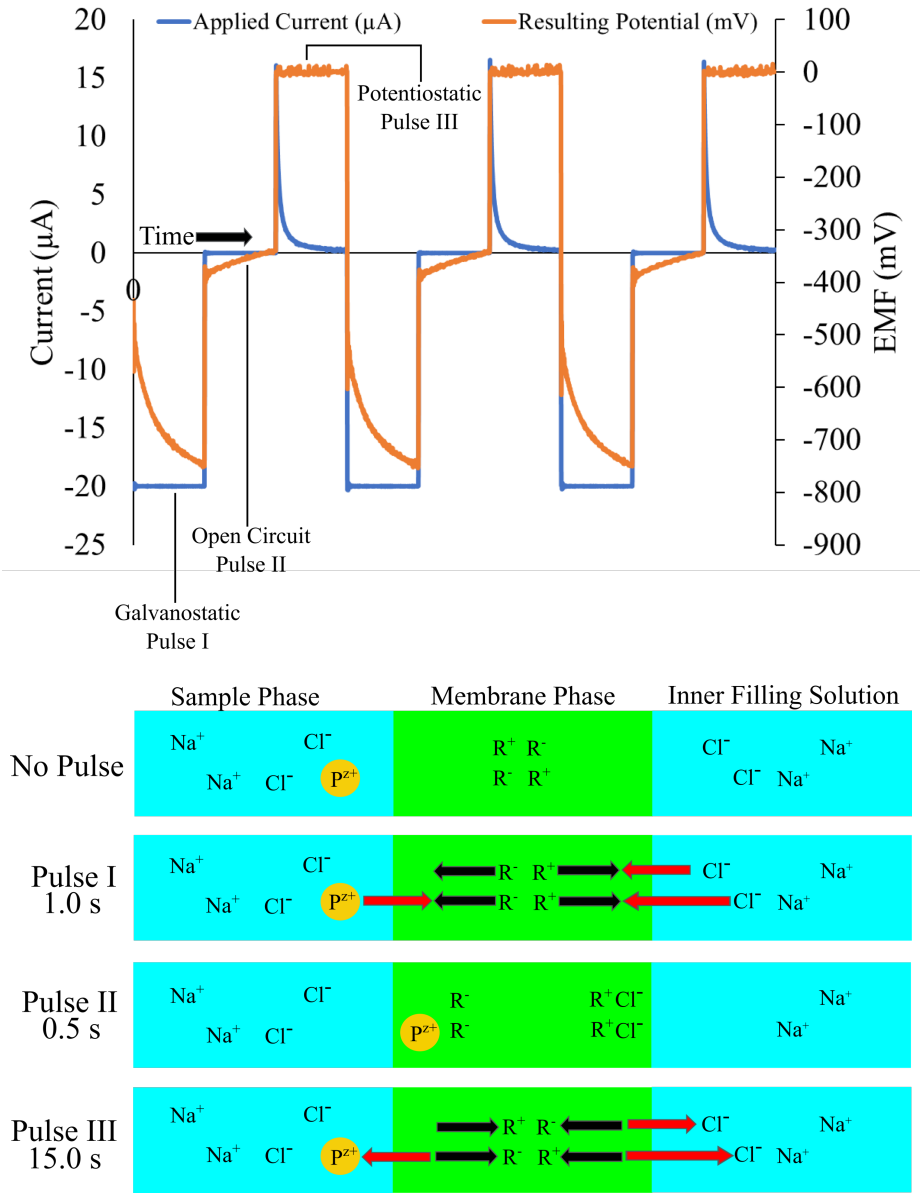

Figure 1.4. Current/resulting potential vs. time trace for a polycation detection experiment using the pulstrode detection system (top) and diagram of the polarization achieved using three electrical pulses and the resulting ion-exchange mechanism which occurs at the sample-membrane interface and the membrane-inner filling solution interface (bottom).

The first membrane composition developed for these reversible polyion sensors consisted of PVC, NPOE, and the $TDDA^+$ – $DNNS^-$ neutral lipophilic salt.[42–45] When compared to single-use polyanion sensors, these reversible sensors employed lipophilic $DNNS^-$ in place of chloride as the counterion to $TDDA^+$ so that no significant ion-exchange or extraction of polyions into the

membrane could occur in the absence of the galvanostatic pulse. This membrane, when integrated into a macroelectrode body, was shown to be capable of detecting polycations using a cathodic current pulse. However, the original sensors of this type were incapable of detecting polyanions (e.g., heparin) when applying an initial anodic current pulse due to the $TDDA^+$ species in the membrane phase which cannot form tight ion-pairs with polyanions (due to steric hindrance).[20] This limitation was overcome by using $TDMA^+$ in place of $TDDA^+$ as the counterion to $DNNS^-$ in the membrane phase.[46] With this neutral lipophilic salt in the membrane, which contained a 1:2 ratio by weight of PVC:NPOE, heparin could successfully be extracted during the anodic current pulse to yield significant negative potential changes when measured during the subsequent open circuit pulse period that were directly proportional to the heparin concentration in the sample phase.

Initial efforts in reversible polyion-sensitive ISEs made use of macroelectrode bodies with appropriately formulated membranes fixed in place at the distal end. Such polyion sensors are reliable and robust but are not considered low maintenance devices amenable to large-scale production. The need for an internal Ag/AgCl RE immersed in a similar electrolyte solution on the backside of the sensing membrane layer is unappealing for creating clinically useful devices. As such, Perera et al.[47] successfully fabricated the first all-solid-contact, fully reversible polyion-sensitive ISEs capable of detecting polycations. This technology was applied to the monitoring of peptidase activities.[48] Although it is reasonable to expect that the detection of polyanions can be achieved using the same all-solid-contact electrode by simply replacing $TDDA^+$ with $TDMA^+$, this has not yet been demonstrated using all-solid-contact reversible polyion sensors. There have also been a number of additional studies that have served to expand the range of reversible polyion detection including the detection of polyanionic contaminants in heparin,[49] protease activities (e.g.,

trypsin, chymotrypsin, and thrombin),[50,51] protamine digestion *via* immobilized thermolysin within a column[52], heparin with protamine permselective membranes,[53] protamine *via* flow chronopotentiometry,[54] and heparin in undiluted human blood using thin layer coulometry.[55] Ion transfer voltammetry[56] is another noteworthy method of reversible polyion detection which is fully discussed in Section 1.4 below.

1.4 Voltammetric Polyion-Sensitive Electrodes

Ion transfer across the interface between two immiscible electrolyte solutions (ITIES) is a phenomenon that has developed into a viable means by which polyions can be detected. However, earlier studies of ion transfer reactions across the ITIES focused on small ion transfer reactions (e.g., alkylammonium cations). Techniques used in these early studies of ion transfer across the ITIES have included chronopotentiometry,[57–59] polarography with an electrolyte dropping electrode,[60–63] cyclic voltammetry,[63–66] and convolution potential sweep voltammetry.[67–71] As interest in ion transfer across the ITIES grew, Taylor and Girault[72] showed that a stable ITIES can be supported at a micropipet tip. Cyclic voltammetry performed using the resulting micro-ITIESs overcame the significant IR drop attributed to the resistance of the organic phase in macroscopic ITIESs targeted at studying ion transfer reactions. Facilitated ion transfer reactions at the ITIES studied by Koryta[64] was later adapted to the study of ion transfer reactions at a micro-ITIES supported at a micropipet tip.[73]

Despite the initial focus of small ion transfer across the ITIES, macromolecular species eventually became a worthwhile target. A number of early studies of macromolecular species using ion-transfer voltammetry included the study of Nafion adsorption/anion transfer,[74] cation binding to DNA,[75] and the adsorption/cation transfer of poly(diallyldimethylammonium chloride)

(PDADMACl) and polyethylenimine.[76] These early polyion sensing studies demonstrated the applicability of ion-transfer voltammetry to the study of polyion behavior at the ITIES and subsequently paved the way for further development in the sensing of biomedically relevant polyions.

Amemiya et al.[56] further applied ion-transfer voltammetry to the study of protamine transfer across water/nitrobenzene, water/1,2-dichloroethane, and water/1,6-dichlorohexane micro-interfaces; the supporting lipophilic electrolyte in this study was TDDA-TpClPB (ETH 500). Shortly thereafter, Samec et al.[77] demonstrated that cyclic voltammetry can be used to study the ion transfer reactions of heparin at the macroscopic interface between a plasticized PVC membrane and an aqueous electrolyte solution. In the case of heparin,[77] the cation within the PVC membrane acts as an ion-exchanger-type ionophore that facilitates the transfer of polyanionic heparin across the ITIES (i.e., facilitated ion transfer). These two studies laid the framework by which biomedically relevant polyions would continue to be studied using ion transfer voltammetry.

A theoretical framework for describing the transfer of polyions across the micro-ITIES, in terms of the relationship between potential and current, was developed in a number of papers.[78–80] In short, the overall transfer reaction of a polyion across the micro-ITIES is as follows: 1) ion-exchanger adsorption to the interface; 2) formation of the ion-exchanger:polyion complex at the micro-ITIES; and 3) complex adsorption.[79] Based on the assumption that complexation between the ion-exchanger and the polyion is the rate-determining step and that the complexation between the ion-exchanger and the polyion is electrochemically irreversible such that only the forward or backward reaction is considered at a given time, the relationship between the potential and

Table 1.1. Expressions for steady state voltammograms for three different mass transfer conditions where E is the potential, $E_{1/2}$ is the half-wave potential, R is the universal gas constant, T is the temperature, α is the transfer coefficient, n is the charge number of the polyion, F is the Faraday constant, i is the steady-state current, and i_d is the diffusion-limited steady-state current. Equations were collected from Ref. 79.

Component (i) controlling mass transfer	**Analytical expressions for steady state voltammograms**	**Type of micropipet electrode**
Polyion diffusion	$E = E_{1/2} + \frac{RT}{\alpha_i nF} \ln\left(\frac{1 - i/i_{d,i}}{i/i_{d,i}}\right)$	Organic-filled
Ion-exchanger diffusion	$E = E_{1/2} + \frac{RT}{\alpha_i nF} \ln\left(2^{s-1} \frac{\left(1 - i/i_{d,i}\right)^s}{i/i_{d,i}}\right)$	Water-filled
Ion-exchanger:Polyion complex diffusion	$E = E_{1/2} - \frac{RT}{\alpha_i nF} \ln\left(\frac{1 - i/i_{d,i}}{i/i_{d,i}}\right)$	Water-filled

resulting current in a voltammetric experiment can be modeled using the three expressions shown in Table 1.1.[79]

The first studies on protamine ion transfer across the micro-ITIES used ETH 500 as the lipophilic supporting electrolyte. However, similar to earlier potentiometric studies of protamine sensing,[35,36] $DNNS^-$ was also suggested as another ion-exchanger capable of facilitating ion transfer of protamine across the micro-ITIES.[79] The voltammetric response of heparin was later examined using different lipophilic ion-exchangers in the organic phase.[81] Octadecyltrimethylammonium was determined to be the ideal ion-exchanger for heparin detection using cyclic voltammetry across the micro-ITIES and was therefore successfully applied to heparin detection in undiluted sheep blood with a detection limit of 0.13 U/mL using stripping voltammetry.[81] Ion transfer voltammetry has also been extended to the development of a solid-supported membrane electrode.[80,82]

A rotating disk electrode in which a plasticized PVC membrane doped with hexadecyltrimethyammonium was used in the amperometric detection of heparin with detection limits reaching 1.4 U/mL in aqueous samples. In this case, the ion-exchanger:heparin complexation is driven by the redox reaction of 1,1′-dimethylferrocene.[82] Gold-supported heparin-sensitive voltammetric ISEs have also been developed using poly(3-octylthiophene) as the ion-to-electron transduction layer between a gold solid support and heparin-sensitive PVC layers.[80] This solid contact ISE was characterized by studying the transfer of ClO_4^- and further applied to heparin detection for which a 0.005 U/mL detection limit for heparin in saline was obtained.[80] Electrochemical recognition of low molecular weight heparin was also demonstrated by cyclic voltammetry on the micro-ITIES using different quaternary ammonium species.[83] These ion transfer studies of polyionic heparin and protamine have provided a means by which the mechanism of polyion extraction and adsorption occurring at the ITIES can be applied to a variety of electrochemical applications that rely on charge transfer across an interface.

1.5 Polyion-Sensitive Ion-Selective Optodes (ISOs)

The development and application of optical sensing devices (polyion-sensitive ISOs) was a logical progression in polyion sensing technologies. ISOs were first introduced in the late 1980s and early 1990s to detect simple cations/anions and were composed of polymeric films doped with lipophilic ionophores and pH chromoionophores.[84–87] It was not until the detection of polyions *via* potentiometric sensors was first introduced in the early 1990s that optical detection of polyions received attention.[88,89]

Initially, simple spectrophotometric measurements were used to detect heparin in plasticized PVC films containing 3-hydroxy-4-(4-nitrophenylazo)phenyl octadecanoate (ETH

2412) (a pH chromophore), $TDMA^+$ (ion-exchanger; with chloride as initial counterion), and DOS (plasticizer).[88] Each of these components was dissolved in tetrahydrofuran (THF) and spin-coated onto glass slides to form thin sensing films. Similar to the original single-use polyanion-sensitive ISEs, a polyanion can be favorably extracted into the thin film to electrostatically interact with $TDMA^+$ (see Figure 1.5). These sensor films differ from conventional single-use electrochemical sensor films in that protons are co-extracted into the membrane to maintain electroneutrality within the optical sensor bulk. The coextraction of the proton and subsequent protonation of the indicator

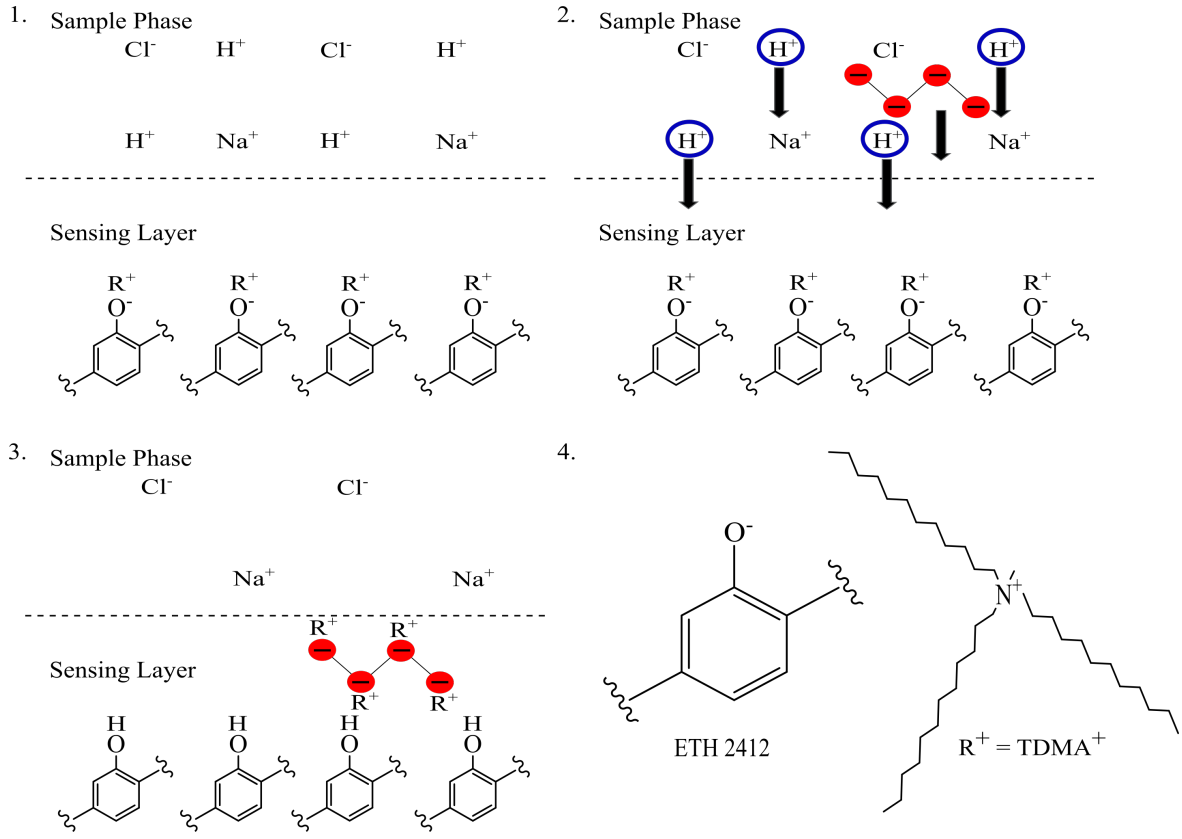

Figure 1.5. Coextraction mechanism for a polyanion-sensitive ISO responding to a generic polyanion. The sensing membrane is subjected to an aqueous buffer solution (1). A polyanion is subsequently injected into the buffer solution at a given concentration (2). The polyanion partitions into the sensing layer by forming cooperative ion-pairs with the $TDMA^+$ species (R^+) while four protons (H^+) are coextracted into the sensing layer to maintain electroneutrality within the sensing membrane bulk (3). The chemical structures of the membrane components are defined (4).

species (i.e., ETH 2412) results in a change in absorbance measurable with a spectrophotometer.[88] This coextraction can be described by a simple equilibrium reaction with an equilibrium constant, $K_{eq,\ heparin}$[88]

$$K_{eq,heparin} = \frac{[\text{ETH 2412} - \text{H}]^z_{org}[\text{TDMA}^+_z - \text{Heparin}]_{org}}{[\text{Heparin}^{z-}]_{aq}[\text{TDMA}^+]^z_{org}[\text{H}^+]^z_{aq}[\text{ETH 2412}]^z_{org}} \quad (1.4)$$

Optical detection of polycationic protamine was achieved using 2',7'-dichlorofluorescein octadecyl ester (DCFOE) (chromoionophore), PVC/Tecoflex polyurethane, and DOS (plasticizer).[89,90] Thin film sensors were fabricated on glass slides in the same fashion as the optical sensing films for heparin described above. In these sensing films, the synthetic chromoionophore exists in its protonated form. Once the sensing film is subjected to a sample containing polycations (e.g., protamine), the polycation is extracted into the film and participates in an ion-exchange reaction with the proton of the chromoionophore with an equilibrium constant, $K_{eq,protamine}$[89]

$$K_{eq,protamine} = \frac{[\text{Protamine} - \text{DCFOE}]_{org}[\text{H}^+]^z_{aq}}{[\text{Protamine}^{z+}]_{aq}[\text{H}^+\text{DCFOE}^-]^z_{org}} \quad (1.5)$$

Protons are ejected into the sample phase to maintain electroneutrality within the membrane bulk resulting in a change in film absorbance. This change in absorbance can also be monitored using a spectrophotometer.[89] One of the most significant differences in these polycation- vs. polyanion-sensitive optodes is that the chromoionpohore used in the polycation-sensitive optodes functions as both the charge carrier and the chromoionophore. Conversely, the charge carrier and the chromoionophore (i.e., $TDMA^+$ and ETH 2412, respectively) for the polyanion-sensitive optodes are separate molecules.

Polyion-sensitive ISOs continued to be developed in their use in a more efficient microtiter plate format [91–93] as well the introduction of the first polymer-/plasticizer-free polyion-sensitive

ISOs (also discussed later in the work). In short, polymer-/plasticizer-free polyion-sensitive ISOs were fabricated by dissolving chromoionophore I and $DNNS^-$ in cyclohexanone and subsequently printing the mixture onto cellulose paper using an inkjet printer.[94] These sensors were capable of direct polycation detection and indirect polyanion detection using protamine, heparin, and PPS as model polyions.[94] Optical protamine detection has also been demonstrated in nanoscale dimensions.[95] Here, optical detection of protamine takes place within nanospheres of DOS and pluronic F-127 doped with the lipophilic ion-exchanger, $DNNS^-$, and Ox blue, a synthetic chromoionophore.[95] The sensing mechanism for these ion-sensitive optical nanosensors can be described as facilitated protamine extraction *via* $DNNS^-$ and the concomitant deprotonation of Ox Blue; deprotonation of Ox Blue results in a blue color.[95]

1.6 Approaches to Cosmetic/Industrial Polyion Sensing

Polyion sensing has historically been largely restricted to biological/biomedical analytes (e.g., heparin/protamine,[8–10,34,36,41] fucoidan,[22] PPS,[21] etc.). Therefore, there have been few polyion sensing applications targeted toward polyions used in fields outside biology/biomedicine. While there has been some studies that examine more industrial polyions such as PDADMACl[76], there is still a significant void in sensing application and development for industrial polyions. This void can facilitate a new wave of polyion sensing applications as the matrices in which cosmetic/industrial polyions are used can present a number of disparate challenges when compared to more traditional aqueous or blood samples. Despite the historical practice of relegating polyion analysis to biological/biomedical applications, polyions are well known to be used in industrial water treatment[96,97] and personal care and/or laundering products (e.g., lotions, conditioners, fabric softeners, etc.).[98–104] Most applications within these fields make use of polycations, which are

commonly polyquaterniums (PQs). These salts are macromolecular compounds that contain multiple positive charges along their polymeric backbone. These charges are derived from quaternary ammonium functionalities and exist independent of the solution pH (strong polycations).[96,105] PQs are widely used in contact lens solutions,[106–109] shampoos,[98–100,110,111] sunscreens,[104,112–115] biocides/algaecides,[116–118] pool additives that improve the feel of swimmers' skin,[119] and as routine flocculating agents in water treatment processes.[97]

With increasing interest in these compounds over the last several decades, there has been a need to develop new devices and methodologies capable of detecting, characterizing, and quantifying these compounds. Single-use electrochemical polyanion-sensitive ISEs were first applied to indirect PQ detection in a surfactant/PQ separation and quantification method (see Chapter 1 of this dissertation).[120] This study demonstrated how single-use polyion-sensitive ISEs can be employed to detect a given PQ species (i.e., PQ-10). Both single-use and reversible polyion-sensitive ISEs were later applied to the broad detection, quantification, and characterization of a variety of PQs and drew attention to the universal PQ detection capabilities of these sensors through indirect detection.[121,122] The PQs targeted in these studies included PQ-2, PQ-6 (also known as PDADMACl), and poly(2-methacryloxyethyltrimethylammonium) chloride (PMETAC) in addition to PQ-10 (see Figure 1.6 for chemical structures). Each of these PQs possess different structures, charge densities, diffusion behaviors, etc. which can present challenges when developing a universal method/device that can accurately and reproducibly characterize such PQs through direct detection. In particular, charge density is an important characteristic of PQs and can be defined as the amount of charge per unit mass of the polymer.

$$\frac{\text{Number of Charged Groups}_{\text{monomer}}}{\text{Formula Weight}_{\text{monomer}}} \times 1000 = \text{mEq/g} \tag{1.6}$$

Table 1.2. Charge densities of four different PQs

PQ Species	Charge Density (mEq/g)
PQ-6	6.18
PQ-2	5.36
PMETAC	4.81
PQ-10*	1.87

*Calculation based on a single degree of ethoxylation for variable "x" in Fig. 6.

This value is customarily expressed as mEq/g and can be calculated using Equation 1.6.[123,124] Different PQs exhibit varying charge densities which allow some PQs to be used more preferably than others for a given application (see Table 1.2 for charge densities of the four PQs shown in Figure 1.6). For example, PQ-6 has a relatively high charge density while PQ-10 has a relatively low charge density.[125,126] As such, it is well known that PQ-6 is routinely used as a flocculating agent in water treatment processes and PQ-10 is routinely employed in cosmetic products.[97,126,127] Further, the number of PQ species commercially available is much larger than the four discussed in these studies [101,123,128] As polyion-sensitive ISE responses are governed, in part, by the charge density of the analyte polyion (i.e., lower charge densities generally translate to lower total ΔEMF signals), the response generated by a PQ species with a significantly low charge density might generate an inadequate ΔEMF signal. However, by performing a titration using a titrant polyanion with a known electrochemical sensor response profile (e.g., DS) and using a polyanion-sensitive ISE as a detector, a universal method for PQ detection can be developed for many PQ species (using both single-use and pulstrode ISEs) provided the PQ species in question tightly binds the titrant polyanion in solution. Figure 1.7 demonstrates this titration method by showing typical titration curves generated by titrating increasing concentrations of PMETAC with polyanionic DS in a buffer solution. Despite indirect detection being the preferred method of detection for PQs, Ferguson and Meyerhoff [121,122] (see Chapters 3-4 of this dissertation) were still able to demonstrate

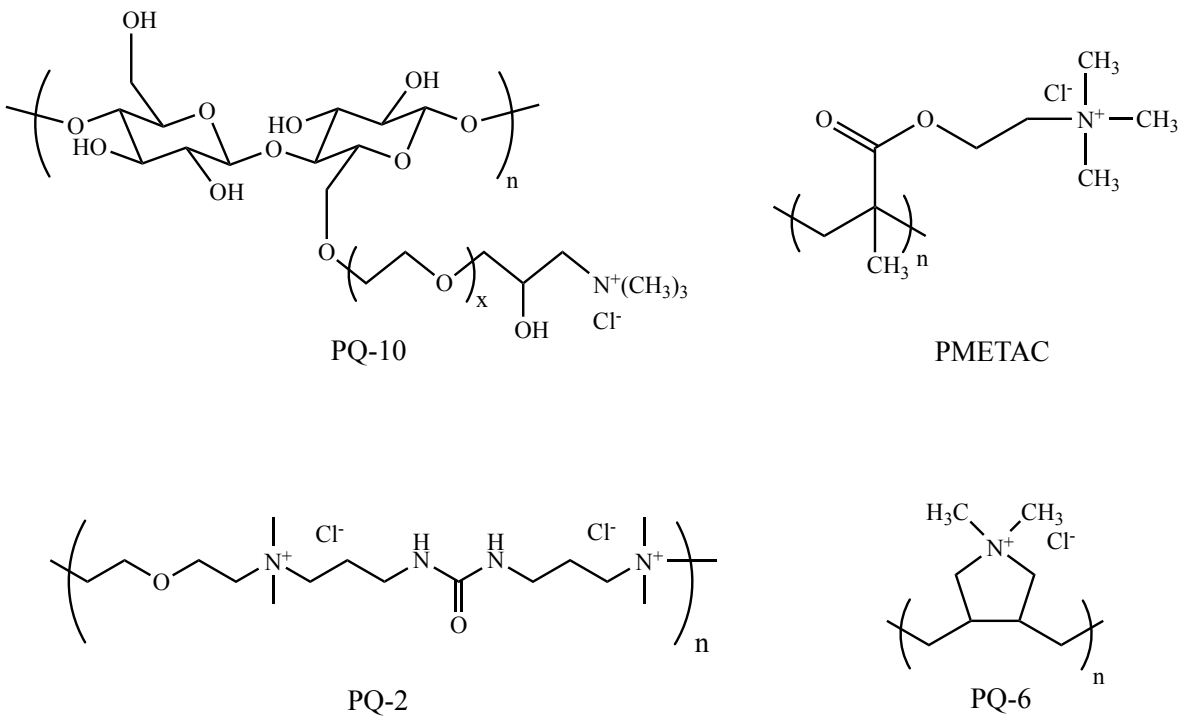

Figure 1.6. Chemical structures of four different PQ species detected by polyion sensors.

that some PQs can be directly detected by single-use and fully reversible polyion-sensitive ISEs. However, the large size of the error bars in the direct detection dose-response curves for single-use sensors, the relatively close proximity of the total ΔEMF responses for three of the four tested PQs for single-use sensors, and the high concentrations of PQ-10 required to generate significant ΔEMF signals using fully reversible sensors reinforces the idea that the indirect method is the preferred mode of PQ detection.

As mentioned above, polycations can also be readily detected with polyion-sensitive ISOs.[89,91,129] Very recently, the development of a polymer-/plasticizer-free polyion-sensitive ISO was reported by Wang et al.[94] These new polyion-sensitive ISOs are markedly different from previously described polymer membrane-based polyion-sensitive ISOs in that they require no polymer matrix (e.g., PVC) or plasticizer. Indeed, the lipophilic ion-exchanger and pH chromoionophore are co-adsorbed as an ultrathin layer on the surface of cellulose paper which

preserves the porosity of the paper and facilitates the addition of a pre-dried buffer reagent into the sensor strip to control proton activity when detecting polyions in real samples.[94] This work focused on detecting protamine and heparin, as well as detecting protease activities using protamine as a substrate.[94]

Similar ISOs were applied to cosmetically/industrially relevant polycations by using polymer-/plasticizer-free polycation-sensitive ISOs to characterize the optical response of four PQs (see Chapter 5 of this dissertation).[130] In this study, polycation-sensitive ISOs were fabricated by inkjet printing a mixture of H^+DNNS^- and chromoionophore I directly onto cellulose paper; smaller polycation-sensitive ISOs were cut from the larger parent membranes and used as individual polycation sensors.[130] Prior to the addition of a sample containing a PQ species to these polycation-sensitive ISOs, chromoionophore I exists in its protonated form within the sensing layer because the proton is donated by the H^+DNNS^- species.[94] When a PQ species is introduced to the

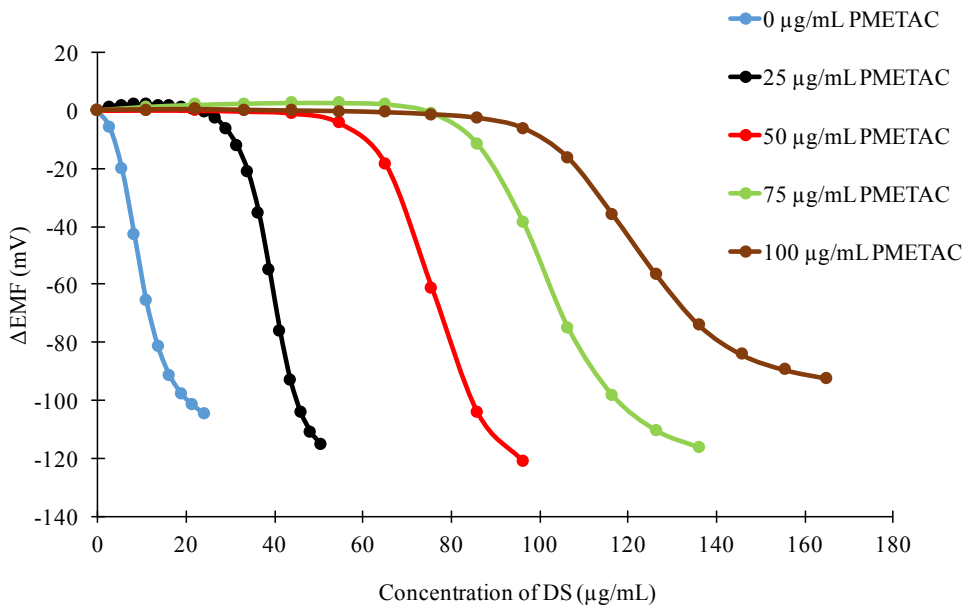

Figure 1.7. Conventional potentiometric titrations of increasing concentrations of PMETAC in phosphate buffered saline using DS as a titrant polyanion and a single-use polyanion sensor as a detector. The polymer membrane contained 66 wt. % PVC, 32.5 wt. % DOS, and 1.5 wt. % TDMAC. The phosphate buffered saline was diluted from a stock 500 mM phosphate buffer, pH 7.4, with 500 mM NaCl solution (50x PBS).

polycation-sensitive ISO, the PQ can form multiple complexes with $DNNS^-$ which requires chromoionophore I to release its proton to the sample phase, resulting in an observable change in hue from blue to purple.[130] This hue change can be easily measured by using a hue analysis software such as Color Mate in conjunction with a common iPhone.[131] Color Mate is an iPhone application that analyzes the hue within an area of a photograph taken by a camera; typically the camera built into an iPhone. The program can analyze the color within a selected area of the photograph and assign a numerical value to the selected area. These values can be used as analytical signals.

Ferguson et al.[130] demonstrated that polyion-sensitive ISO fabrication does not have to be relegated to the use of common polymer matrices (e.g., PVC) or plasticizers. The ability of mechanical supports and sensing components to be optimized as desired and the ability to dry reagents on/within these sensors broadens the number of applications to which these sensors can be applied, including cosmetic/industrial polyions. A key advantage is that these sensors do not require expensive laboratory equipment to detect the analytical signals because a simple smart-phone equipped with Color Mate[131] software was used to detect hue changes on photos taken of the sensing site.[130] This feature enables such technology to be employed for in-field analyses in studies such as water treatment in less developed regions of the globe.

1.7 Summary

Over the last few decades polyion sensing *via* electrochemical and optical detection devices/methods have proven to be applicable to the detection of many biological, biomedical, cosmetic, and industrial polyions. These technologies have introduced a new field of polyion measurement and characterization that can overcome many challenges inherent to polyion analyses

(e.g., high molecular weights, lack of UV absorption at higher wavelengths, etc.).[132] Further, potentiometric analysis of polyions using polyion-sensitive ISEs (reversible and single-use sensors) have bypassed the need for redox-active sites that are customarily required for redox dependent voltammetric analysis.[133]

All-solid-contact polyion-sensitive ISEs are a frontier of ongoing research that has the capability of enabling more automated, compact, and high throughput screening of polyions. There have been relatively few advancements to date in this area.[47] Using such approaches could allow for more cost- effective device fabrication, which could translate into higher numbers of analytical devices being produced in large batch fabrication processes. Paper-based ISEs for small ion detection have shown promise in the detection of Na^+, K^+, NO_3^-, Cd^{2+}, Ag^+ , NH^{4+}, and H^+ during the last few decades.[134–138] Analogous paper-based polyion-sensitive ISEs should expect similar development. PNA modified nanopores used for potentiometric detection of nucleic acids have also been recently proposed and hold promise in their application to other polyelectrolytes.[139] Paper-based devices are especially attractive for a variety of applications and have demonstrated superiority in many aspects of fabrication/implementation including low cost, mechanical flexibility, air permeability, wicking capability, etc.[140]

Finally, new polyion-sensitive ISOs have also been successfully demonstrated, enabling polyion analysis without need for an external RE. These ISOs incorporate the use of inexpensive sensing components and/or mechanical support layers allowing these optical sensors to be cost-effective, disposable units.[141] Much more work in this area is anticipated, especially relating to in-field measurements of polyions and in point-of-care applications for monitoring hospitalized patients (e.g., levels of PQs in municipal water and heparin in whole blood, respectively).

1.8 Research Statement

There is a significant lack of research and development targeted toward the sensing of cosmetic/industrial polyions. Indeed, as there are a number of biomedically important polyions (e.g., protamine, heparin, etc.) it is understandable that initial polyion sensing efforts have been targeted at the detection of these polyions in matrices such as human blood. However, there are many cosmetic/industrial polyions that are useful in a variety of applications which have not been explored using electrochemical and optical polyion sensing technologies. Chapter 2 begins to address this by reporting on the application of potentiometric polyion-sensitive ISEs to the indirect detection of PQ-10. This work focuses on the separation of sodium lauryl sulfate (SLS) from solutions that contain PQ-10 and SLS *via* an anion-exchange resin. The remaining PQ-10 is then quantified by performing potentiometric titrations and employing single-use polyanion-sensitive ISEs as detectors; dextran sulfate (DS) serves as the polyanionic titrant. This work was published in *Analytical Methods* (Ferguson, S.A.; Wang, X.; Meyerhoff, M.E. *Anal. Methods* **2016**; 8 (29): 5806-5811).

Chapter 3 demonstrates the ability of single-use polyion-sensitive ISEs to be applied to both direct and indirect polycation sensing. To accomplish this both single-use polycation-sensitive (for direct detection) and polyanion-sensitive (for indirect detection) ISEs were constructed and applied to the detection of four different PQ species. Polyanion-sensitive ISEs were also shown to be capable of indirectly detecting PQ-6 in water samples obtained from the Ann Arbor, MI drinking water treatment plant. This work was published in *ACS Sensors* (Ferguson, S.A.; Meyerhoff, M.E. *ACS Sensors* **2017**; 2 (2): 268-273). Chapter 4 reports on the application of fully reversible polyion-sensitive ISEs to the direct and indirect detection of PQ species and also demonstrates the ability of these reversible sensors to be incorporated into a semiautomated flow-

through system (i.e., flow-injection analysis (FIA) system). In this same work, PQ-6 was shown to be capable of quantification in recreational swimming pool water. This work was published in *ACS Sensors* (**Ferguson, S.A.**; Meyerhoff, M.E. *ACS Sensors* **2017**; 2 (10): 1505-1511).

Chapter 5 reports on the application of the first polymer-/plasticizer-free polyion-sensitive ISOs to the direct detection of PQ species. These paper-based devices are reported to be capable of detecting four PQs in aqueous media using an iPhone camera as a detector. Indirect polyanion detection using these same paper-based devices is also demonstrated in this chapter as well as their application to the detection of PQ-6 in recreational swimming pool water. This work was published in *Analytical Sciences* (Ferguson, S.A.; Wang, X.; Mahoney, M.; Meyerhoff, M.E. *Anal Sci.* **2018**; 34 (1): 45-50).

Chapter 6 reports on the development and application of the first paper-based all-solid-contact polyanion-sensitive ISEs to the detection of four polyanions. These sensors circumvent the need for an inner filling solution in contact with the polymeric sensing membrane in early polyion-sensitive ISE designs and represent the construction of truly disposable single-use polyion-sensitive ISEs. Finally, Chapter 7 provides the conclusions and implications of this dissertation research and provide direction and guidance for future polyion sensing research and development.

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CHAPTER 2

Detecting Levels of Polyquaternium-10 (PQ-10) *via* Potentiometric Titration with Dextran Sulphate and Monitoring the Equivalence Point with a Polymeric Membrane-Based Polyion Sensor

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2.1 Introduction

Polymeric quaternary ammonium salts (polyquaterniums) represent a class of polyions that have found increasing use in industrial and cosmetic applications around the world (e.g., industrial flocculation agents and as conditioners in personal care products).[1–3] These compounds are known to be toxic to certain aquatic species.[4] Further, manufacturers need to add appropriate amounts of these species to personal care product formulations to elicit the desired effects (e.g., thickening agents for hair, etc.).

It is often found that polyquaterniums (PQs) of low charge density are used in personal care products (e.g., shampoos).[5] More specifically, the quaternized hydroxyethylcellulose compound, PQ-10 (see Figure 2.1a), is commonly present within shampoo formulations containing

* All experiments, data analysis, and figure construction for this chapter were performed by Stephen A. Ferguson. Xuewei Wang was responsible for assisting in the development of my understanding and application of polyion-sensitive ion-selective electrode (ISE) technology. Mark E. Meyerhoff was the principal investigator for the project. P&G and the MBSTP at the U-M (NIH grant number T32 EB005582) funded the entirety of the study.

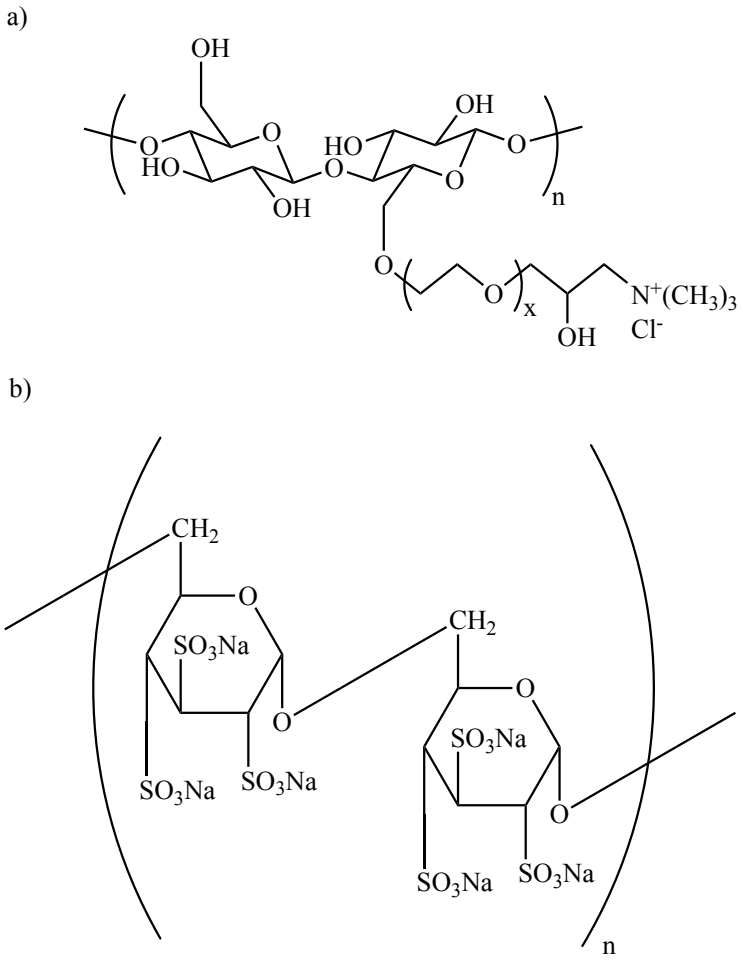

Figure 2.1. (a) Chemical structure of PQ-10. (b) Chemical structure of dextran sulphate (DS).

anionic surfactants.[6,7] In these formulations PQ-10 is solubilized with relatively high concentrations of anionic surfactants (e.g., sodium lauryl sulfate (SLS)).[6] After dilution, which normally takes place during the bathing process, the anionic surfactant:PQ-10 ratio reaches a level at which an anionic-polycationic complex precipitates. This facilitates the desired hair-conditioning effect to take place, allowing hair to lie flat. For this reason, PQ-10, and other PQs, are also known as antistatic agents.[5,6] As such, it has become increasingly important to quantitate these compounds using analytical techniques.

Since PQ-10 is a polycationic species, the application of electrochemical polyion-sensitive polymeric membrane ion-selective electrodes (ISEs), introduced in the early 1990s,[8–10] for the

detection and quantification of this compound in various shampoo formulations should prove useful. However, anionic surfactants have been known to give large potentiometric responses with poly(vinyl chloride) (PVC)-based ISEs.[11,12] Since these surfactants are customarily found in shampoo formulations, this poses an analytical challenge in the application of this polyion sensing technology to the quantification of PQ-10 in such samples. Therefore, to provide a reliable and reproducible detection method for the measurement of PQ-10 in such samples with polyion-sensitive ISEs, it will be necessary to remove SLS from solutions containing both polycationic PQ-10 and anionic surfactant in a way that does not alter the level of PQ-10 present. This chapter discusses the application of a simple ion-exchange treatment method, initially introduced by Weber and Kuter[13] for the separation of SLS from solutions containing protein and urea, to effectively remove SLS from solutions containing both PQ-10 and SLS. The resulting solution is then acidified and subsequently titrated with dextran sulphate (DS) (see Figure 2.1b) and monitored using a single-use polyanion-sensitive polymeric membrane ISE as the detector to provide equivalence points directly proportional to PQ-10 levels.

2.2 Experimental Section

2.2.1 Chemicals and Reagents

DS sodium salt from Leuconostoc (MW 500,000), glacial acetic acid, and double junction reference electrode inner and outer filling solutions were purchased from Fisher Scientific (Waltham, MA). SLS (97.98%) was obtained from Calbiochem (Billerica, MA) and tridodecylmethylammonium chloride (TDMAC) was purchased from Polysciences (Warrington, PA). Bis(2-ethylhexyl) sebacate (DOS), high molecular weight PVC, tetrahydrofuran (THF),

Dowex® monosphere anion-exchange resin (hydroxide form) (584 µm particle size), and sodium hydroxide pellets were products of Sigma-Aldrich (St. Louis, MO).

2.2.2 Membrane Preparation and EMF Measurements

All experiments were performed at ambient temperature (22˚C). Polymeric membranes were cast using the method described by Craggs et al.[14] Each membrane was batch fabricated using membrane cocktails containing 800 mg of ingredients dissolved in 8 mL of THF. Each batch of membrane cocktail contained 66% PVC, 32.5% DOS, and 1.5% TDMAC (w/w) dissolved in 8 mL of solvent. All ingredients were dissolved in anhydrous THF and allowed to mix overnight. Once cocktails were completely solubilized in THF, each batch was poured into a glass casting ring (8.75 cm i.d.) placed on a homemade glass slide (14 x 14 cm) and allowed to evaporate at room temperature overnight. After the solvent was evaporated, small discs (5 mm i.d.) were cut using a cork borer and integrated into Ostec electrode bodies (Oesch Sensor Technology, Sargans, Switzerland). The inner filling solution consisted of a 10 mM NaCl electrolyte. Potentiometric responses were measured against an external Ag/AgCl double junction reference electrode. Potential values were recorded by averaging the last 10 equilibrium EMF values three minutes after each DS injection. The solutions being titrated were stirred via stir bar for the duration of the titration.

2.2.3 Ion-exchange of SLS in PQ-10/SLS Mixtures and Subsequent Manual or Automated Potentiometric Titration of PQ-10 with DS

All mixtures of PQ-10/SLS as well as the DS titrant were dissolved in a background electrolyte of 10 mM NaCl. Five solutions of 5% (w/v) SLS were dissolved in 10 mM NaCl. Known amounts of PQ-10 were also added to four of the five SLS solutions (0.1, 0.2, 0.3, and

0.4% (w/v), respectively). These solutions were allowed to dissolve completely before being diluted to mark. Each solution was subsequently diluted 1:50 with 10 mM NaCl to provide a working solution for extraction and measuring the PQ-10 concentration.

Dowex resin was exhaustively washed prior to use in the conventional manner described by Weber and Kuter.[13] If resin was not used immediately in an ion-exchange process, the beads were stored in distilled water. Approximately 120 mL of anion-exchange resin was packed into an appropriately sized glass column. Working solutions were prepared via 1:50 dilutions using 10 mM NaCl as a diluent and allowed to flow through the column. Four fractions of each mixture were collected. After the fractions were collected, 50 mL aliquots of each ion-exchange resin treated sample were used for titrations. Alternatively, thick suspensions were used for ion-exchange as describe by Weber and Kuter.[13] Suspensions were allowed to mix on a rotator for no less than 30 minutes for complete ion-exchange prior to sample titration. Each aliquot was acidified prior to titration via addition of 50 μL of glacial acetic acid. The working and reference electrodes were allowed to obtain a stable baseline EMF value prior to the start of the titration. For manual titrations, the 50 mL aliquots were subsequently titrated with DS in 10 mM NaCl and the titration was followed using single-use polyion-sensitive polymeric membrane ISEs as described previously for titrations of polycationic protamine with polyanionic heparin.[9] Regeneration of the column resin was achieved by exhaustively flushing the beads with a 0.1 M NaOH solution prior to the next resin treatment. For automated titrations, 50 mL aliquots of increasing concentrations of PQ-10 in 10 mM NaCl were titrated with DS in 10 mM NaCl using a dual-syringe infusion/withdrawal pump (Cole-Parmer, Vernon Hills, Illinois); there was no surfactant present in these samples initially, therefore, ion-exchange was not necessary.

2.3 Results and Discussion

2.3.1 SLS Removal via Ion-Exchange

As SLS is a strong interfering species when using polyanion-sensitive polymeric membrane ISEs, it is necessary to remove SLS from the sample prior to detection. Figure 2.2 illustrates the effective removal of SLS from an aqueous solution containing both SLS and PQ-10. A 100-fold dilution of a mixture of 0.5% (w/v) PQ-10 and 5% (w/v) SLS solution was prepared using 10 mM NaCl as a diluent in both the stock and diluted samples. The diluted sample was then subjected to ion-exchange using a suspension of anion-exchange resin as described by Weber and Kuter.[13] After the ion-exchange process the resin was allowed to settle to the bottom of the

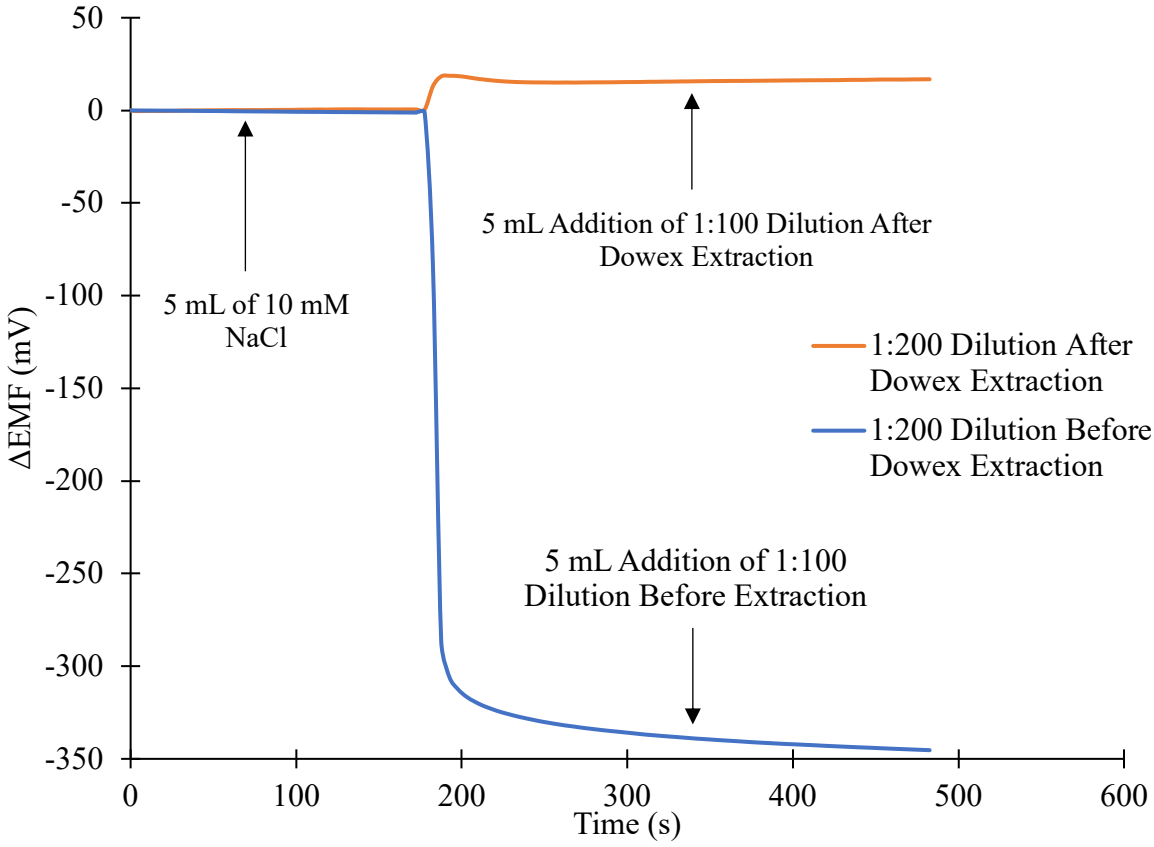

Figure 2.2. EMF response of a single-use polyanion sensor to 5 mL of a 1:100 dilution of extracted vs. non-extracted samples of a 0.5%/5% PQ-10/SLS solution after dilution.

container and a 5 mL aliquot of the supernatant was added to a 5 mL solution of 10 mM NaCl background electrolyte into which a single-use polyion sensor had previously obtained a stable baseline EMF value; this resulted in a total 200-fold dilution of the stock 0.5%/5% PQ-10/SLS mixture. This process was also completed using a 100-fold dilution of the stock 0.5%/5% PQ-10/SLS solution which did not undergo an ion-exchange process. As shown in Figure 2.2, the EMF responses of the working electrode toward the diluted sample that was not subjected to ion-exchange resulted in a large negative EMF response due to the presence of SLS within the sample. In contrast, the diluted sample that was treated with the ion-exchanger resulted in the EMF remaining at the baseline value established in the 10 mM NaCl background electrolyte. This indicates that the SLS concentration was reduced to a level at which there is negligible interference to the TDMAC-based polyanion sensor membrane.

2.3.2 Verification of Non-Adsorption of PQ-10 to Ion-Exchange Resin

Prior to quantification of the remaining PQ-10 in solution it is necessary to ensure that PQ-10 does not interact with the anion-exchange resin after a sample is treated. Figure 2.3 depicts how PQ-10 is not adsorbed to the anion-exchange resin. This figure shows two potentiometric titrations of identical solutions of 25 μg/mL PQ-10 in 10 mM NaCl. One aliquot of this solution was not subjected to the anion-exchange resin while the other aliquot was subjected to the resin followed by addition of glacial acetic acid to acidify the sample; a 50 mL aliquot of sample subjected to anion-exchange resin was treated with 50 μL of glacial acetic acid. Each of the resulting solutions were titrated with DS until an endpoint was obtained and first derivative plots were employed to determine the equivalence point for each titration. The equivalence points generated by these two titrations resulted in only a 4.3% difference. These data suggest that there is no significant

adsorption of PQ-10 to the anion-exchange resin during the ion-exchange process. However, it should be noted that the total ΔEMF generated from the acidified ion-exchanged sample showed a marked increased at the endpoint when compared to the sample that was not subjected to anion-exchange resin. This suggests that the addition of a weak acid such as acetic acid can increase the total ΔEMF of this potentiometric titration of PQ-10 with DS, indicating that at lower pH values, the polyanion sensing membrane exhibits more significant response to the DS polyanionic species. This is in agreement with findings reported by Schwake et al.[15] in which potentials for DOS plasticized PVC membranes doped with various quaternary ammonium species were reported for different sodium salts, including sodium acetate.

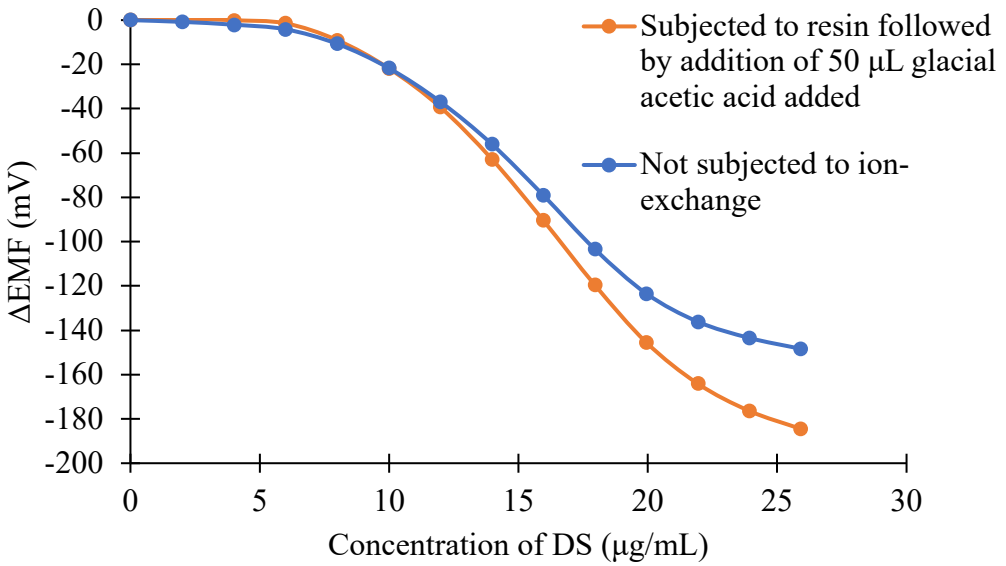

Figure 2.3. Non-adsorption verification of PQ-10 onto the anion-exchange resin. One solution was subjected to anion-exchange/acidification and one was unperturbed. Equivalence points for both aliquots are quite similar.

2.3.3 Quantification of PQ-10 via Potentiometric Titration

An appropriate method was needed to effectively detect the remaining PQ-10 in solution after SLS removal. As there are two specific methods (indirect and direct detection) that can be used for this task, it was necessary to determine which would provide appropriate and reliable data for PQ-10 quantification. Because PQ-10 is known to have a charge of approximately +1 per disaccharide unit resulting from the quaternary ammonium group attached to the hydroxyethyl

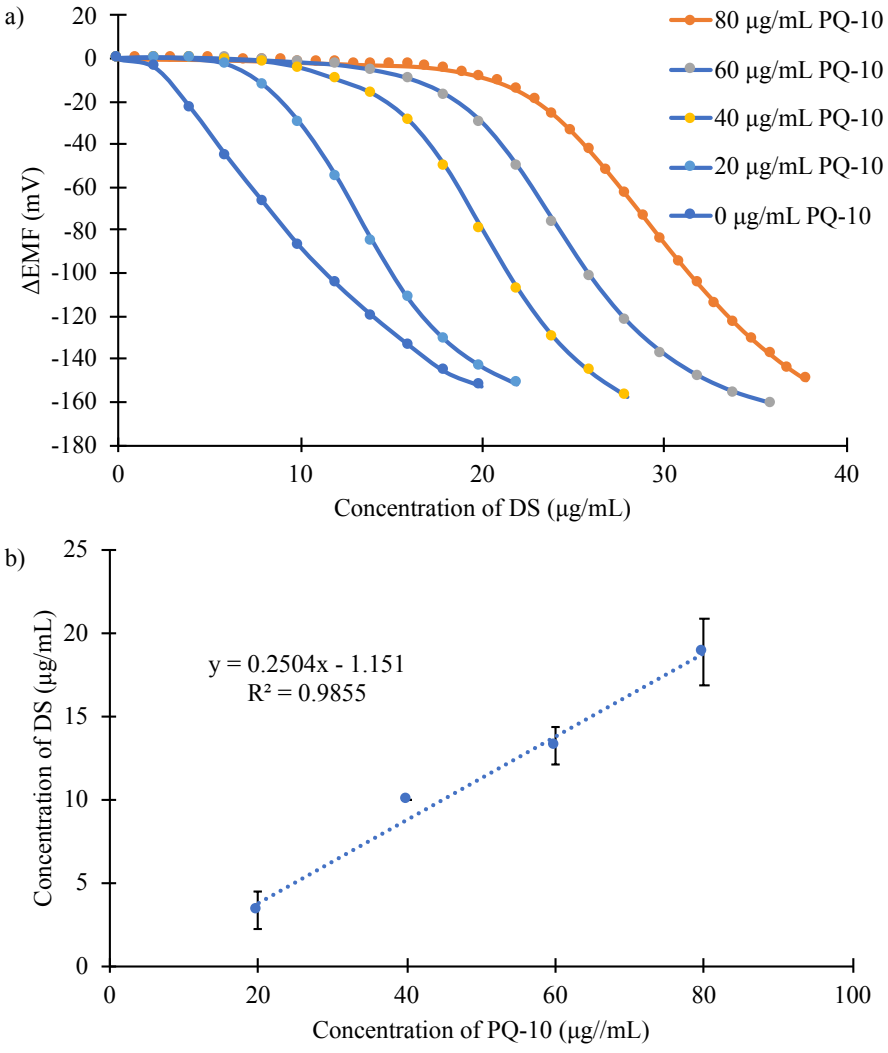

Figure 2.4. (a) Titration curves of PQ-10 at various concentrations with DS as titrant in 10 mM NaCl background electrolyte. The data points for each curve represent the average ΔEMF from three electrodes placed in the same PQ-10 solution. (b) Calibration curve from data under (a).

side-chain[16,17] it can be expected that PQ-10 would yield a relatively small total ΔEMF (compared to the more characterized responses of polyionic species heparin and protamine) if direct detection with a polycation sensing membrane electrode based on dinonylnapthalene sulfonate (DNNS) were to be employed.[18] Hence, in this work, the titration of PQ-10 with the high charge density DS species and use of the polyanion sensor described above was pursued. Figure 2.4a illustrates typical titrations of varying levels of PQ-10 using DS as the titrant as monitored by TDMAC-based polyanion sensing membrane electrodes after SLS removal. It is demonstrated that as more PQ-10 is present in the original sample (with SLS also initially present), after removing the SLS, the equivalence points of the titration curves obtained are directly proportional to the concentration of PQ-10 in the samples. This indicates that even with the low charge density of the PQ-10 species, a very strong ion-pair formation between DS and PQ-10 occurs within the aqueous phase. Figure 2.4b illustrates the calibration curve obtained when plotting the equivalence points of the titrations in terms of amount of DS required (using first derivative plots to obtain equivalence point values)

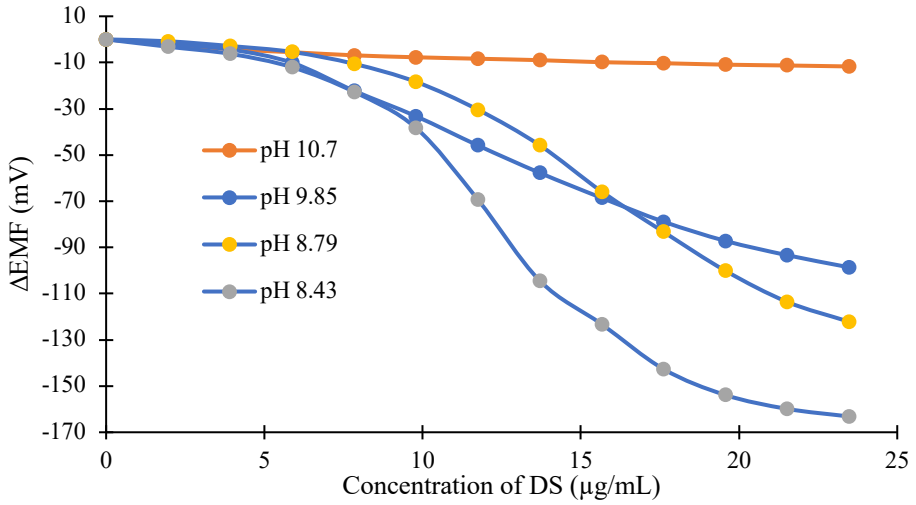

Figure 2.5. Hydroxide ion interference to TDMAC-doped polyion-sensitive polymeric membrane ISEs at various pH values. Each curve is a single titration monitored by three electrodes simultaneously placed in the same solution.

vs. PQ-10 in the sample, illustrating the expected linear relationship. Using this method, PQ-10 levels as low as 20 µg/mL can be detected.

Figure 2.5 shows the effect of the hydroxide ion levels on the polyanion-sensitive polymeric membrane. Solutions of 25 µg/mL PQ-10 in 10 mM NaCl were prepared with known amounts of NaOH. This resulted in 25 µg/mL PQ-10 in 10 mM Cl^- solutions at four different pH values. As illustrated in the plot, each polyanion response shows a decreased negative slope as the response deviated further from the super-Nernstian response reported by Ma et al.[9] These data suggest that the pH of the extracted solution will need to be closely monitored after the anion-exchange process. If the pH of the solution is too high, the pH can be adjusted using an appropriate acid which contains an anion that would not interfere significantly with the polyanion sensing membrane. The initial potential of the electrode is controlled by the Cl^- level in the NaCl electrolyte solution.[19,20] In addition, as notated in Table 2.1, it was determined that the starting potentials of each titration decreased as pH increased. This demonstrates that excess hydroxide begins to affect the sensor prior to the addition of polyanionic DS. To circumvent this issue, one might try to use a suitable buffer. While phosphate buffered saline (PBS) is an ideal buffer for biological applications, the use of an anion-exchange resin (hydroxide form) causes the released hydroxide concentration to exceed the buffer capacity of conventional concentrations of PBS. For samples

Table 2.1. Starting potentials of various solutions of 25 µg/mL PQ-10 dissolved in 10 mM NaCl at different pH values. These starting potentials are averages of the three electrodes placed in the same electrolyte solution.

pH	Average Equivalence Point (Concentration of DS (µg/mL))	Starting Potential (mV)
8.43	13.05	142.16
8.79	15.66	132.38
9.85	10.45	101.10
10.71	NA	-14.96

containing variable amounts of SLS the amount of anion-exchange resin will need to be adjusted accordingly. This will result in variable pH values and increase the potential of exceeding the buffer's capacity to maintain a constant pH value. Hence, adjustment of the pH of the final PQ-10 sample to a relatively fixed, low pH value after the anion-exchange process to remove SLS will help ensure that the total EMF changes observed for the titrations remain relatively similar.

2.3.4 Automated Titrations of PQ-10 via Syringe Pump-Based Potentiometric Detection Method

To increase the sample throughput of the titration method of PQ-10, it would be advantageous to use a syringe pump that would facilitate continuous titrant injection into the sample in addition to reducing random error related to injection placement and/or timing (i.e., injections were made every three minutes for the manual titrations described above). Figure 2.6a illustrates initial results from efforts targeted at this approach. Syringe pump titrations were performed using samples with increasing concentrations of PQ-10 in 10 mM NaCl; no surfactant was initially present in these samples and anion-exchange was therefore not performed. Here, each increasing concentration of PQ-10 is shifted further to the right. This resulted in the expected equivalence points as higher concentrations of DS are added. Results are in excellent agreement with the manual PQ-10 titrations performed above. Figure 2.6b illustrates the calibration data of the resulting equivalence points calculated *via* first derivative plots from each titration curve. In addition, the total ΔEMF for these samples was markedly lower than the acidified effluents collected after treatment with anion-exchange resin. This further illustrates how the addition of a weak acid such as acetic acid can increase the total ΔEMF for a given polyanion when these electrochemical polyanion-sensitive polymeric membrane ISEs are used.

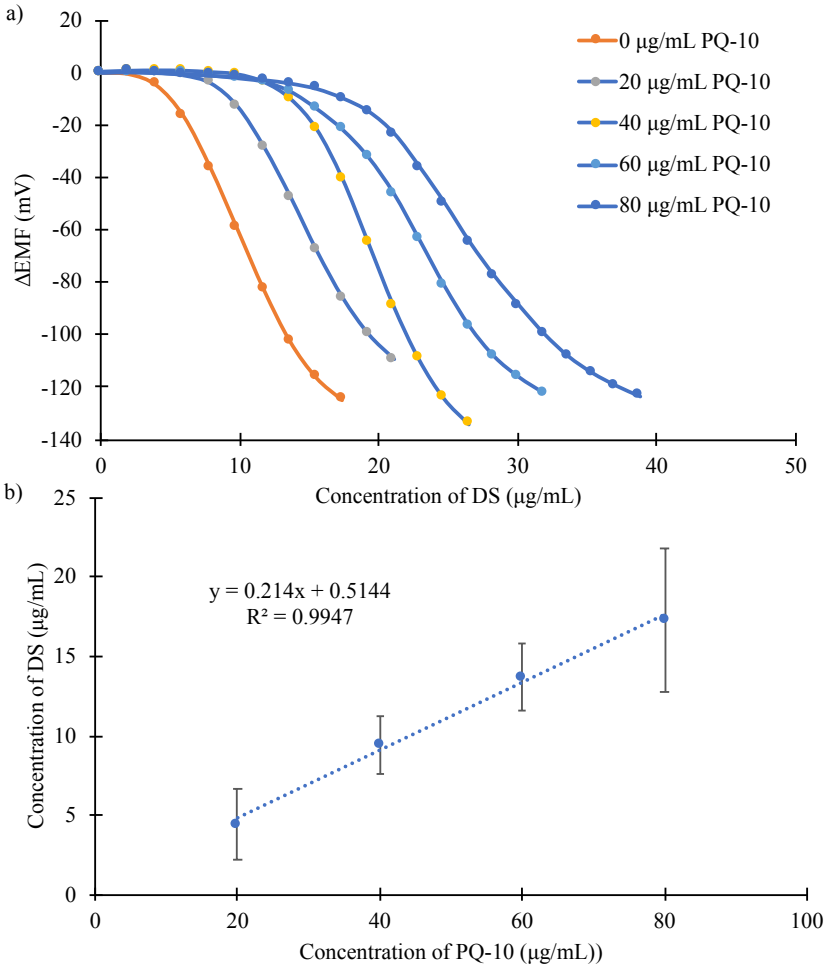

Figure 2.6. Syringe pump-assisted titrations of increasing concentrations of PQ-10 with DS as titrant. A flow rate of 200 µL/min. of a 0.5 mg/mL titrant solution of DS was used for sample volumes of 50 mL. The data points for each curve represent the average ΔEMF from three electrodes placed in the same PQ-10 solution. (b) Calibration curve from the data under (a). The standard deviations of the 20, 40, 60, and 80 µg/mL PQ-10 samples are 2.19, 1.85, 2.10, and 4.49, respectively.

2.4. Conclusions

Over the last 20+ years, potentiometric polyion sensing has grown to be a useful analytical method to detect and quantify different polyions in various chemical environments.[9,18,21] More recently, potentiometric polyion sensing has also been further developed through its application to

potentiometric aptasensing,[22,23] biomedical analyses without the use of moving components,[24] and the reversible potentiometric detection of biomedically relevant polyions.[25-28] At the same time, PQ-10 has found increasing use in cosmetic applications such as shampoo formulations for its ability to act as a conditioner to aid in repairing damaged hair. As PQ-10 is a polycationic species that forms strong complexes with polyanions, as shown here, the use of a polyanion-sensitive electrode for its quantification via an indirect titration method is a promising new approach that can be applied in various quality control applications in industrial settings. Also, the addition of the ion-exchange method described here is shown to be effective in the reduction of any potential SLS interference. This enables the reliable detection of PQ-10 via the potentiometric titration of PQ-10 using DS as a titrant polyanion.

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CHAPTER 3

Characterization and Quantification of Polyquaterniums *via* Single-Use Polymer Membrane-Based Polyion-Sensitive Electrodes

Ferguson, S.A.; Meyerhoff, M.E. *ACS Sensors* **2017**; 2 (2): 268-273 *

3.1 Introduction

Polymeric quaternary ammonium compounds (polyquaterniums) are a class of macromolecules that have been used extensively in environmental, industrial, and cosmetic applications. Some specific applications include flocculation in water treatment processes, fiber strengthening agents in paper production, and as conditioners in personal care products (e.g., shampoos, lotions, conditioners, etc.).[1,2] Polyquaterniums (PQs) contain multiple positive charges (i.e., polycations) which are generally derived from organo-ammonium groups. These ammonium groups can differ in their connectivity to the polymeric backbone and their positive charge is not a function of sample pH.[3]

PQs and/or other polyelectrolytes also have biomedical and sensing applications such as their use in electrospun nano-fiber mats,[4] humidity sensors,[5] and hydrogels.[6] The biomedical

* All experiments, data analysis, and figure construction for this chapter were performed by Stephen A. Ferguson. Mark E. Meyerhoff was the principal investigator for the project. P&G and the MBSTP at the U-M (NIH grant number T32 EB005582) funded the entirety of the study.

applications of PQs also extend to microbiology, where they are commonly used as antibacterial agents and are even found in commercial contact lens solutions.[7,8] Since the use of these compounds is growing rapidly it is important to have attractive detection methods for these species to assist in their quantification and/or characterization in various samples.

At present, detection of PQs presents significant challenges as they generally possess high molecular weights, have no electrochemically active domains, and do not exhibit significant absorbance at wavelengths > 260 nm.[9] As such, new methods for PQ measurements are needed. One possibility is the use of polymer membrane-based (e.g., plasticized poly(vinyl chloride) (PVC)) polyion-sensitive electrodes where certain lipophilic ion-exchangers (e.g. tridodecylmethylammonium chloride (TDMAC) for polyanion sensing and sodium dinonylnaphthalene sulfonate (NaDNNS) for polycation sensing) are incorporated within the polymeric film. These types of ion-exchanger-doped polyion sensors were introduced in the early 1990s as a means to detect and quantitate various polyionic species, especially the polyanionic anticoagulant, heparin.[10–12] Interestingly, the electromotive force (EMF) responses toward polyions exhibited by these sensors is much greater than predicted by the Nernst equation for highly charged polyionic species.[13,14]

Further, the observed response to polyions occurs over a narrow concentration range of the polyion of interest.[13] This differs from the behavior of conventional ion-exchanger-based ISEs that target small ionic species (e.g., Cl^-, K^+, Na^+, Ca^{2+}, etc.). In addition, conventional ISEs require higher analyte ion concentrations in the sample phase before a significant potential change is observed compared to polyion sensors.[14] This is because conventional ISEs contain the analyte ion of interest equilibrated within the polymeric membrane phase prior to the membrane being subjected to a sample; this is usually accomplished by pre-soaking the membrane in a solution of

the target analyte ion or simply having the analyte ion present as the counter ion to the lipophilic ion-exchanger when the membrane cocktail is first formulated.[15] In contrast, polyion sensors contain no polyion within the membrane prior to a measurement. This facilitates a nonequilibrium partitioning of the polyion from the sample phase into the membrane as it exchanges for the counterion of the lipophilic ion-exchanger and subsequent formation of a cooperative ion-pair between the ion-exchanger and the analyte polyion.[14]

To our knowledge, the application of polyion sensors for the detection and quantification of PQs has only recently been pursued.[16] In this prior work PQ-10, a polycationic hydroxyethylcellulose,[6] was the target polyion that was detected *via* potentiometric titration using a polyanion sensor with a PVC membrane doped with TDMAC. Interestingly, because sodium dodecyl sulfate (SDS) needed to be removed from the model cosmetic samples targeted in that work *via* an anion-exchange resin (hydroxide form), the pH of the sample phase needed to be adjusted using acetic acid.[16] Therefore, if the sample were to contain more significant concentrations of SDS it would become necessary to add higher concentrations of acetic acid to neutralize the excess hydroxide ion in solution. This requires that the pH of the sample be closely monitored.

In this chapter, we report on the electrochemical responses of polycation and polyanion sensing membrane electrodes to four different PQs (PQ-2, PQ-6, PQ-10, and poly(2-methacryloxyethyltrimethylammonium) chloride (PMETAC)) (see Figure 1.6) in a buffered background electrolyte using direct and indirect (titrimetric) detection methods. This buffered system provides an environment in which the pH is controlled more reliably. It will also be demonstrated that each target PQ species can be detected and quantified at ppm levels using direct or titrimetric detection methods. The titrimetric method is also applied to the detection of PQ-6

spiked into water samples provided by the Ann Arbor, MI, drinking water treatment plant. This approach could prove useful in potentially detecting excess PQ-6 in the effluent found after a secondary flocculation step of the drinking water treatment process that involves the use of PQ-6.

3.2 Experimental Section

3.2.1 Chemicals and Reagents

Glacial acetic acid, sodium chloride, and the double junction reference electrode outer filling solution were purchased from Fisher Scientific (Waltham, MA). Sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate were purchased from Amresco, Inc. (Solon, OH). TDMAC was a product of Polysciences (Warrington, PA). Bis(2-ethylhexyl) sebacate (DOS), 2-nitrophenyl octyl ether (NPOE), high molecular weight PVC, anhydrous tetrahydrofuran (THF), PQ-2, PQ-6, PQ-10, and PMETAC were purchased from Sigma-Aldrich (St. Louis, MO). NaDNNS was a gift provided by King Industries (Norwalk, CT).

3.2.2 NaDNNS- and TDMAC-Doped Single-Use Sensors: Membrane Preparation and Integration into Macroelectrode Bodies

The 10 mM phosphate buffer, pH 7.7, with 10 mM NaCl (10 mM PBS) used as a diluent and inner filling solution for all experiments was prepared by diluting a 500 mM phosphate buffer, pH 7.4, containing 500 mM NaCl solution (50x PBS), unless otherwise noted. Membranes were cast in the conventional manner described by Craggs et al.[17] Membrane cocktails were batch fabricated using a total of 800 mg of total ingredients. NaDNNS-doped sensors were composed of 49.5% PVC, 49.5% NPOE, and 1% NaDNNS (w/w). TDMAC-doped sensors were composed of 66% PVC, 32.5% DOS, and 1.5% TDMAC (w/w). All ingredients were dissolved in

approximately 8 mL anhydrous THF overnight and subsequently poured into a glass casting ring (8.75 cm i.d.) placed overtop a glass slide (14 x 14 cm). Evaporation of the THF occurred overnight to insure complete loss of solvent. Small discs (5 mm i.d.) were cut using a cork borer and integrated into Ostec electrode bodies (Oesch Sensor Technology, Sargans, Switzerland). The inner filling solution for each polyion sensor consisted of 10 mM PBS.

3.2.3 Direct Detection of PQs

To determine the total ΔEMF response of NaDNNS-based electrodes toward each PQ species, dose-response experiments were performed. Three polycation sensors were assembled and placed in a solution of 10 mM PBS; potentials were measured against a double junction Ag/AgCl reference electrode placed in the same solution. A stable baseline was recorded before the start of each dose-response experiment. A dual-syringe infusion/withdrawal pump (Cole-Parmer, Vernon Hills, IL) was used to deliver the desired 0.5 mg/mL PQ in 10 mM PBS at a fixed rate of 288 μL/min. The ΔEMF values were calculated by taking the average of the last 10 pseudo-equilibrium potential values (recorded every 5 s) of each 1 min. interval after pump initiation. Therefore, each ΔEMF value corresponding to a concentration of added titrant represents the ΔEMF value taken at the concentration infused in the solution at each 1 min. interval. The concentration of titrant corresponding to each ΔEMF value is calculated based on the time the average EMF data point was collected and the flow rate that was used during the titration. All solutions were stirred continuously *via* magnetic stir bar.

3.2.4 Indirect Detection (Potentiometric Titration) of PQs

Solutions of increasing concentrations of the desired PQ were made in individual beakers. Each concentration was titrated using dextran sulphate (DS) as titrant and followed by three polyanion sensors (made with TDMAC) placed in the same solution. A stable baseline was recorded when measured against the double junction Ag/AgCl reference electrode on all polyion sensors prior to starting the pump. The same Cole-Parmer dual-syringe infusion/withdrawal pump used for polycation response experiments was used to deliver the titrant at a fixed rate of 288 µL/min. The ΔEMF values were calculated in the same fashion as direct detection experiments. All test solutions were stirred continuously *via* magnetic stir bar.

3.2.5 Verification of Residual PQ-6 in Water Samples

A 49 mL sample of effluent obtained after secondary flocculation at the Ann Arbor, MI, drinking water treatment plant was placed in a beaker (using an appropriate number of 5 mL and 4 mL micropipette additions) in addition to 20 µL of 0.5 M acetic acid. A 1 mL aliquot of 50x PBS was subsequently added to the sample and allowed to mix *via* magnetic stir bar resulting in a 1:50 dilution of the 50x PBS stock solution. A separate aliquot of 10 mM PBS (50 mL) was placed in a separate beaker for titration. Once a stable pH was recorded in each of these solutions the solutions were titrated using 0.5 mg/mL DS in 10 mM PBS delivered by the dual-syringe infusion/withdrawal pump. Each titration was followed by recording the EMF signals of three different polyanion sensors placed in the same solution and potentials were measured against the double junction Ag/AgCl reference electrode placed in the same solution. The ΔEMF values were calculated in the same fashion as direct detection experiments.

3.2.6 Detection and Quantification of Spiked Water Samples

Increasing amounts of PQ-6 dissolved in 10 mM PBS were placed in individual beakers. In addition, 20 µL of 0.5 M acetic acid and 1 mL of a 50x PBS stock solution were added to each of these beakers. Finally, appropriate amounts of effluent obtained after secondary flocculation at the Ann Arbor drinking water treatment plant was added to each beaker to make up the remaining volume necessary to equal a total of 50 mL. This provided a 1:50 dilution of the 50x PBS solution. Once a stable pH was recorded for each sample they were titrated with solutions of appropriate concentrations of DS in 10 mM PBS using the same Cole-Parmer dual-syringe infusion/withdrawal pump to deliver the titrant. The ΔEMF values were calculated in the same fashion as direct detection experiments. Potentials were measured against a double junction Ag/AgCl reference electrode. All experiments were conducted at ambient temperature (22 ˚C).

3.3 Results and Discussion

3.3.1 Direct Dose-Response Toward PQs Using Polycation Sensors

The chemical structure and charge density of a polycation can have a significant effect on the total ΔEMF generated using the NaDNNS-based polycation sensors. Figure 2 displays the observed total ΔEMF responses toward the four PQs depicted in Figure 1 recorded using the NaDNNS-based polycation sensors (average signals from n=3 sensors). Each PQ shows a different total ΔEMF which is indicative of its chemical structure, diffusion coefficient within the membrane phase, its charge density, and other properties. As shown in Figure 2, PMETAC, PQ-6, and PQ-2 exhibit relatively high total ΔEMF responses when compared to PQ-10. The low, total ΔEMF of PQ-10 can be explained by its relatively low charge density.[6]

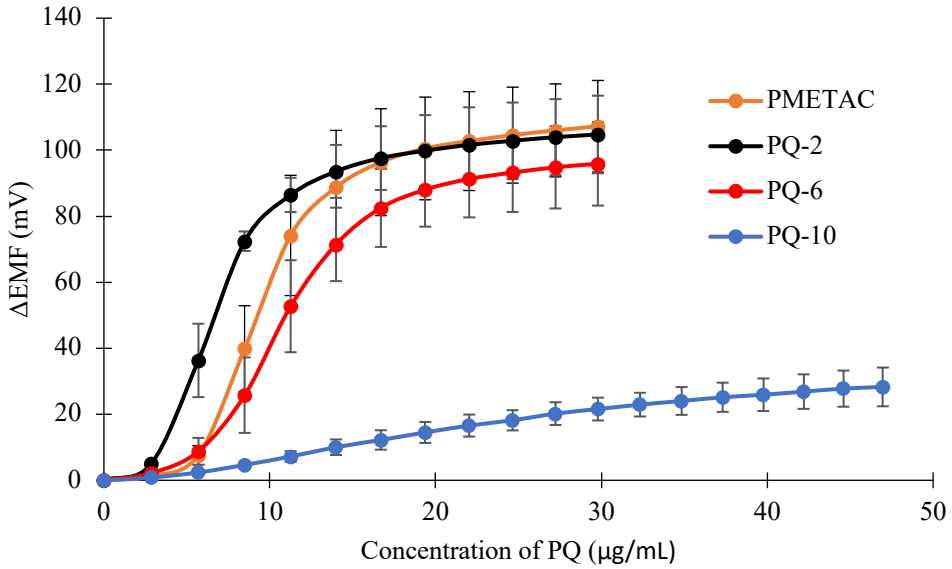

Figure 3.1. Total ΔEMF of four PQs in 10 mM PBS. Each data point represents the average (± s.d.) ΔEMF per concentration of PQ from three different polyion sensors placed in the same solution.

3.3.2 Quantification of PQs via Indirect Titration Method

As PQ species can form tight complexes with negatively charged polyanionic species in solution, another method for their detection is to use a simple potentiometric titration. In this method, a polyanionic species (e.g., DS) is used to titrate the positively charged PQ in solution. Once all of the PQ species have been bound by the DS titrant the excess polyanion will be extracted into the plasticized PVC membrane and exchange for the Cl^- counterions of the $TDMA^+$. This yields a potentiometric titration curve from which an equivalence point can be calculated. This equivalence point is directly proportional to the amount of PQ in the sample.

The membrane composition of the TDMAC-doped polyion sensor for these experiments is significantly different from the DNNS-doped polyion sensor used for direct detection with regard to plasticizer content. This was implemented in accordance with earlier polycation sensor work

based on similar lipophilic ion-exchangers reported by Ramamurthy et al.[18] In this earlier study, it was determined that the optimal membrane composition for protamine – a polycation – sensing was 1.0% DNNS, 49.5% NPOE, and 49.5% polyurethane M48 (w/w). This membrane composition provided the most significant potentiometric response for protamine and was therefore the target for direct detection of all PQ species in this current work.

Figures 3a-d show the titration curves of the four PQs generated using polyanion sensors doped with TDMAC as the detectors. In this figure the titration curves of each increasing concentration of PQ are shifted to the right, thus changing the equivalence point of each curve. Most notably, the shape of the titration curves for PQ-2 are rather different than the shape of the titration curves found for the other three PQ species. This is most likely due to a significantly decreased equilibrium binding constant between DS and PQ-2. Indeed, it has been documented previously that low molecular weight heparin (Lovenox) can bind to polycationic foldamers in blood plasma which serves to reverse the clinical effects of Lovenox.[19] However, some foldamers bind more favorably to Lovenox than others (i.e., some foldamers possess relatively low equilibrium binding constants with heparin). Therefore, for some foldamer-Lovenox titrations the dissociation rate of the foldamer-Lovenox complex may be high enough to provide free Lovenox chains that are able to partition into a polyanion sensitive polyion sensor, eliciting a negative response much sooner than expected.[19] It is likely that this same phenomenon is occurring during the PQ-2 titrations with DS shown in Figure 3c. Further, Figure 4 shows the equivalence points derived from the titration curves displayed in Figures 3a-d. Each curve shows a linear response with limits of detection (3σ) for PQ-6, PQ-2, PMETAC, and PQ-10 being 6.91 μg/mL, 7.71 μg/mL, 9.01 μg/mL, and 21.54 μg/mL, respectively.

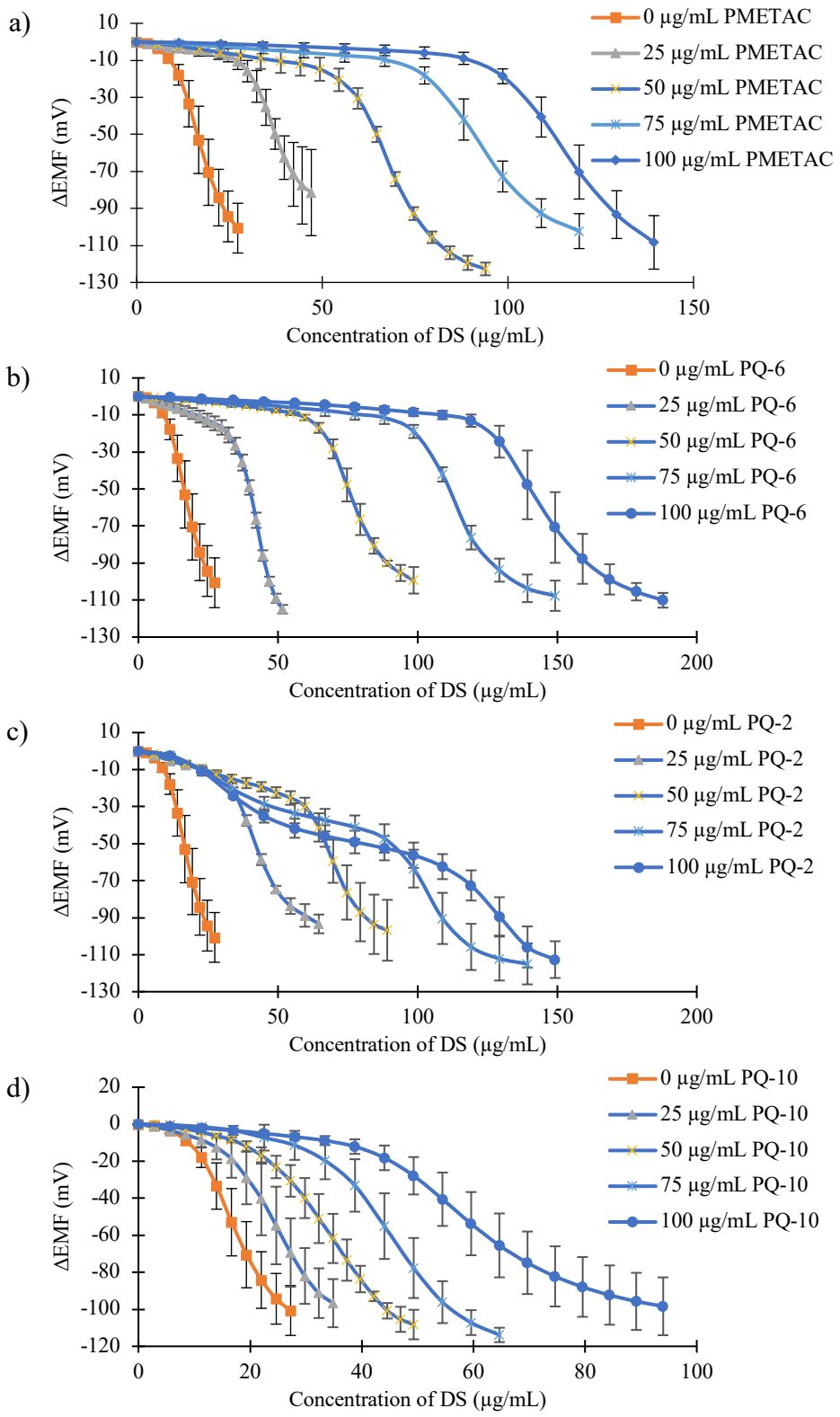

Figure 3.2. Titration curves of (a) PMETAC, (b) PQ-6, (c) PQ-2, and (d) PQ-10 using polyanionic DS as titrant. Each data point represents the average (± s.d.) ΔEMF per concentration of DS from three different polyion sensors placed in the same solution.

The slopes of each of these calibration curves provides insight into the relative charge density of each PQ species. That is, PQ-6 has the highest charge density of the four PQs tested while PQ-10 has the lowest (i.e., it takes more DS to neutralize the cationic charge for a given mass concentration of PQ-6 than PQ-10). The relative low charge density of PQ-10 found through potentiometric titration is in agreement with the degree of quaternary ammonium substitution for the PQ-10 species.[6]

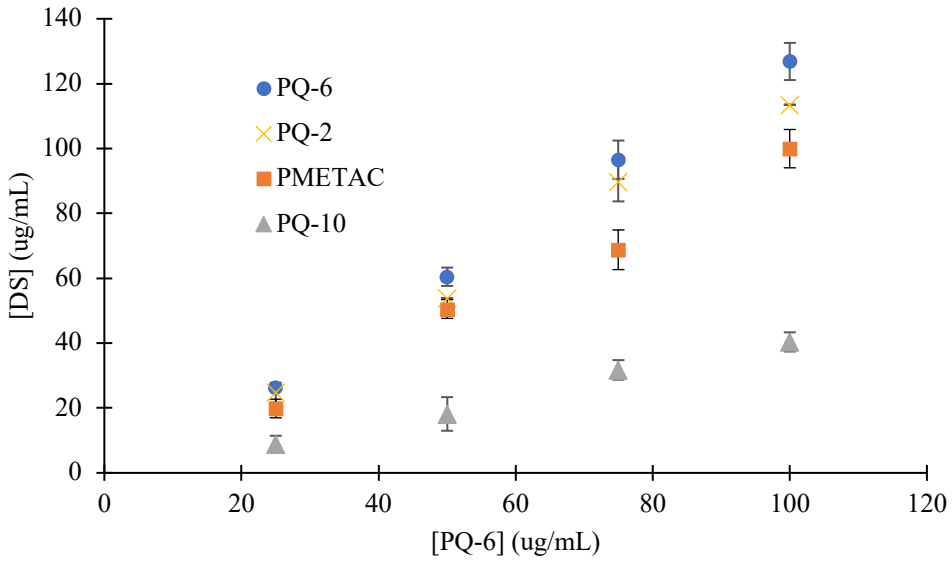

Figure 3.3. Calibration curves derived from the data in Figure 3 *via* first derivative plots.

3.3.3 Detection and Quantification of PQ-6 in Water Samples

The Ann Arbor drinking water treatment plant treats drinking water sourced from the Huron River in Michigan through a series of chemical, physical, and microbiological processes that serve to clarify the water. One of these processes is the addition of PQ-6 to the effluent transported from the primary clarifier where CaO is added as a flocculating agent. PQ-6 is added after the effluent is transported from the primary clarifier and thoroughly mixed. Once the polymer is mixed with the effluent, it is transported to a flocculating system and subsequently moved to a

secondary clarifier where the newly formed flocs sink to the bottom of the reservoir. After flocs collect at the bottom of the reservoir the water is subjected to further treatment. The typical level of PQ-6 used at a drinking water treatment site by NSF® International standards (NSF® once stood for the National Sanitation Foundation) for the purposes of coagulation and flocculation is 25 mg/L.[20] To date, the amount of PQ-6 that might be left in the effluent immediately after flocculation at the Ann Arbor site is not monitored by any analytical method. In addition, most analytical method development has been targeted toward residual PQ detection in the finished product (i.e., water stored for population distribution). There has been some success in targeting lower detection limits of polycations using tannic acid precipitation,[21] fluorescent tagging,[22] and potassium poly(vinyl sulfuric acid) titrations coupled with a Toluidine Blue O indicator dye.[23] However, these methods suffer from limitations such as long standing times for tannic acid precipitation reactions for detecting polycationic polyacrylamides (~1 h), cumbersome polymerization reactions and fluorescent tagging schemes, and buret-based titrations based on colored indicator dyes. In contrast, indirect PQ detection *via* potentiometric titration would provide a more streamlined, facile, and robust method for the quantification of PQ species. To reduce the amount of residual polycation in the drinking water supply it would be beneficial to detect excess polycation immediately after flocculation with PQ-6. This would allow more appropriate amounts of PQ-6 to be added to the effluent without significant reagent waste. This may be important to water treatment processes as water quality and level of contamination found in the water sources may change periodically and is subject to environmental influences. Therefore, the use of polyion sensors could provide an attractive means by which excess PQ-6 in the effluent immediately after flocculation can be monitored to assist in the reduction of unnecessary reagent consumption.

Figure 5 displays the titration curves generated from titrating a 50 mL solution of 10 mM PBS and a 49 mL aliquot of the effluent obtained after secondary flocculation that was treated with 20 µL of 0.5 M acetic acid and 1 mL of the 50x PBS solution (note: pH of solution is typically 7.7 after this process owing to dilution of the ionic strength of the stock concentrated 50x PBS buffer). The addition of acetic acid was done here because the effluent sampled from the Ann Arbor, MI, drinking water treatment plant was found to be approximately pH 9.0. As discussed by Ferguson *et al.*,[16] higher pH values can affect the detection limits of anion-exchanger-based polyanion sensors (see Chapter 2). The effluent sampled from the treatment plant after secondary flocculation is not chemically buffered. Therefore, 0.5 M acetic acid was used to lower the pH value of the effluent to within the buffer capacity range of the 50x PBS buffer. This method of sample preparation was employed to ensure a dilution factor of 50 for the 50x PBS solution. Doing so allowed the final concentration of NaCl in the beaker to be 10 mM in each solution to be titrated.

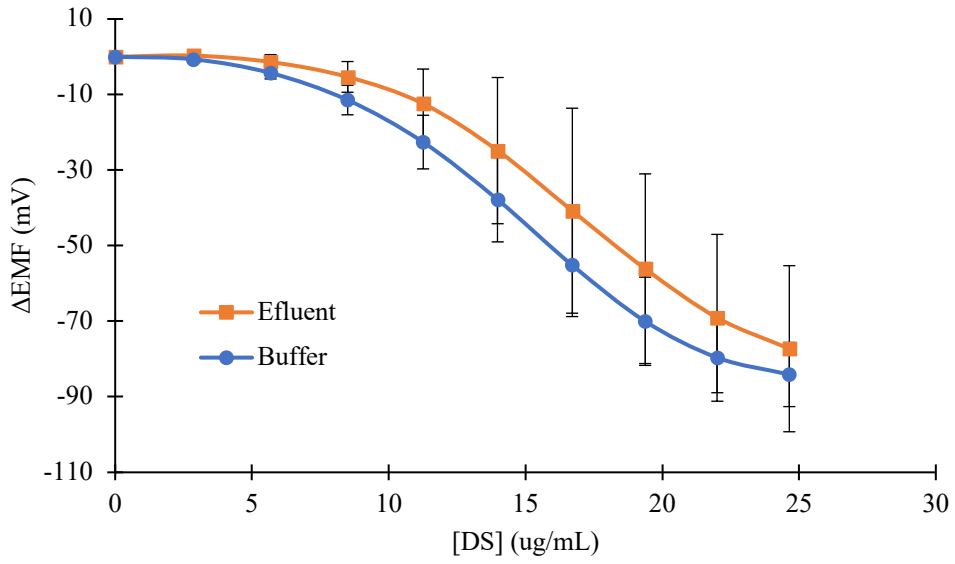

Figure 3.4. Titrations of 10 mM PBS and buffered effluent obtained from the Ann Arbor drinking water treatment plant. Each data point represents the average (± s.d.) ΔEMF per concentration of DS from three different polyion sensors placed in the same solution. The equivalence points calculated *via* first derivative plots of each curve resulted in only a 5.6% difference.

This is the same concentration of NaCl that was used for all other titrations in this work. As there was only a 5.6% difference between the equivalence points generated *via* first derivative plots of each curve (Figure 3.5) it can be inferred that there were negligible amounts of PQ-6 left in the effluent as compared to a solution that contained no PQ-6 (i.e., 10 mM PBS).

Despite the finding that there was negligible PQ-6 in the effluent at the time the effluent was sampled, it is nonetheless important to prove that PQ-6 can be titrated within municipal water samples. This will allow plant employees to determine if adequate or inadequate amounts of PQ-6 have been used to treat the effluent. Figure 6a shows the titration curves of Ann Arbor water samples acquired immediately after the flocculation process at the water plant that were further spiked with increasing concentrations of PQ-6. As the PQ-6 concentration increases the titration curves are shifted further to the right, thus changing the observed equivalence points for each successive curve. In addition, Figure 6b shows the resulting calibration curve generated from the equivalence points of the titration curves found in Figure 6a. This calibration curve shows the expected linear response with an identical slope value to the slope found in the PQ-6 calibration curve generated in 10 mM PBS (see Figure 4). NSF International notes that 25 mg/L is the typical level of PQ-6 employed in coagulation/flocculation processes.[20] Using this method, the limit of detection of PQ-6 in the drinking water effluent matrix is ca. 6.5 μg/mL. Therefore, we believe that this method will provide a reasonable means by which the levels of PQ-6 that remain in the water after the flocculation process can be assessed. It should be noted that if lower detection limits are needed, this can be achieved by altering the composition of the membrane cocktail used to prepare the polyanion sensors.[13]

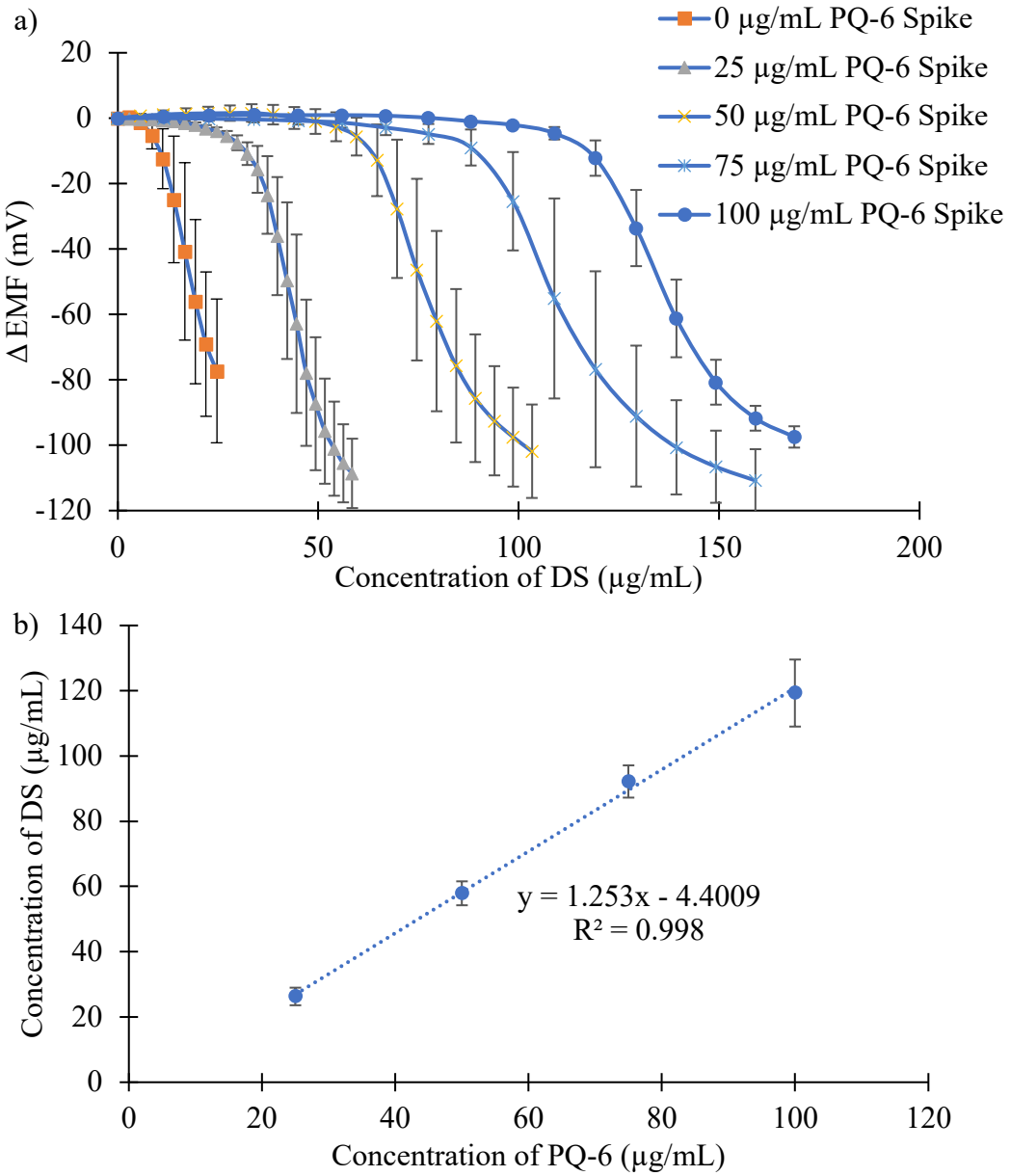

Figure 3.5. (a) Titrations of increasing concentrations of spiked PQ-6 in 49 mL aliquots of effluent buffered with 1 mL of 50x PBS. (b) Calibration curve from the data found in (a). Limit of detection (3σ) was determined to be 6.45 µg/mL PQ-6.

3.4 Conclusions

Herein, we have presented a facile method that can be used for the quantification and characterization of PQs. The method involves the use of lipophilic ion-exchangers (i.e., TDMAC

or NaDNNS) that serve as ion-exchangers for oppositely charged polyionic species. Using this technology PQs can be detected both directly and indirectly. Direct detection of a PQ is conventionally the preferred method; however, because of the low charge density of some PQ species and their varied molecular weights, the resulting total ΔEMF may be too small to be considered analytically useful. Therefore, it is advisable to perform a potentiometric titration (indirect detection) using a polyion sensor doped with a like charged lipophilic ion-exchanger (i.e., TDMAC) combined with a titrant that is known to provide a significant total ΔEMF response (e.g., DS). This will yield a more reliable means by which to detect/quantitate these polymers in various samples. The limits of detection for the indirect detection of these PQs were in the ppm range and the proposed method provides a simple approach by which excess PQ-6 can be detected in drinking water samples.

3.5 References

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CHAPTER 4

Manual and Flow-Injection Detection/Quantification of Polyquaterniums (PQs) *via* Fully Reversible Polyion-Sensitive Membrane-Based Ion-Selective Electrodes

Ferguson, S.A.; Meyerhoff, M.E. *ACS Sensors* **2017**; 2 (10): 1505-1511 *

4.1 Introduction

Cationic polymers (polycations) are employed in many cosmetic,[1,2] water treatment,[3,4] and biomedical products.[5] More specifically, the various polymeric quaternary ammonium salts (polyquaterniums (PQs)) that are used in these applications can possess a wide range of chemical structures, charge densities, binding properties, etc.[6] Because of the diverse nature and applications of these compounds, it has become increasingly important to develop methods by which these polycations can be characterized and quantified. However, since many high molecular weight PQ structures that are widely used exhibit no significant absorbance at wavelengths exceeding 260 nm and have no electrochemically active domains, these compounds are challenging to detect by conventional analytical techniques.[7]

* All experiments, data analysis, and figure construction for this chapter were performed by Stephen A. Ferguson. Mark E. Meyerhoff was the principal investigator for the project. P&G and the MBSTP at the U-M (NIH grant number T32 EB005582) funded the entirety of the study.

To our knowledge, the detection and quantification of PQs by electrochemical sensor technology has only recently been pursued.[8,9] In these previous reports, four different PQs were separated and/or analyzed *via* single-use polymer membrane-based polyion-sensitive electrodes that possessed either a lipophilic cation-exchanger (sodium dinonylnaphthalene sulfonate (NaDNNS)) or anion-exchanger (tridodecylmethylammonium chloride (TDMAC)). The ion-exchanger permits the partitioning of an oppositely charged polyion (e.g., polyanion or polycation) into the plasticized organic membrane phase yielding potentiometric responses. Therefore, for the direct detection of PQ species, $DNNS^-$-doped membrane electrodes were used, while $TDMA^+$-doped membrane electrodes were used for indirect PQ quantification in conjunction with the polyanion, dextran sulfate (DS), being used as the titrant.[10,11]

While single-use polyion sensors provide an effective and sensitive means by which PQs can be detected, the irreversible nature of these devices does not enable this measurement technology to be readily automated. To circumvent this issue for polyion measurements in general, a fully reversible polyion sensing "pulstrode" system was introduced by Shvarev and Bakker.[12] This technology employs a three-electrode system consisting of working, double-junction reference, and counter electrodes that can facilitate the reversible detection of polycations (e.g., protamine). This is achieved by doping a plasticized poly(vinyl chloride) (PVC) membrane with a salt of two oppositely charged lipophilic ion-exchangers (i.e., tetradodecylammonium ($TDDA^+$) and $DNNS^-$) and applying a short-lived (1 s) galvanostatic pulse that would serve to polarize the membrane, allowing the analyte polycation to partition into the membrane to interact with the $DNNS^-$ species yielding a detectable open circuit voltage change, and then subsequently be ejected back into the sample phase using a longer-term (15 s) potentiostatic pulse.[12,13] This technology was later changed to incorporate $TDMA^+$ in place of the $TDDA^+$ species for the reversible

detection of polyanionic heparin.[14] This new fully reversible detection scheme was subsequently incorporated into a flow-injection analysis (FIA) system in which the semi-automated detection of protamine and heparin was achieved.[7]

In this chapter, we describe the adaptation of this pulstrode methodology to the detection of four different PQ species. Specifically, we demonstrate that PQ-2, PQ-6, PQ-10, and poly(2-methacryloxyethyltrimethylammonium) chloride (PMETAC) (see Figure 1.6) can each be detected *via* direct response of the pulstrode in the polycation sensing mode, as well as by an indirect, but more analytically useful, polyanion detection method (with polyanion serving as titrant). PQ-6, in particular, at concentrations in the range of 100-200 ppm, has been found to be useful in improving the feel of hair and skin as perceived by swimmers in recreational water sources (e.g., pools and spas).[15] Levels in spa water are typically higher (e.g., 200 – 400 ppm).[15] In addition, various PQs have found use as sanitizers/algaecides in pool water.[16] As such, we also demonstrate that PQ-6 can be detected and quantified in recreational swimming pool water collected in Ann Arbor, MI. Lastly, we show the ability of PQ-6 to be quantified by using a pulstrode system as a detector in a simple FIA arrangement. The methods described herein could provide universal approaches by which a diverse group of PQs can be characterized and detected in a wide variety of sample types.

4.2 Experimental Section

4.2.1 Chemicals and Reagents

Sodium phosphate (monobasic monohydrate) and sodium phosphate (dibasic heptahydrate) were purchased from Amresco, Inc. (Solon, OH). Sodium chloride and the double junction reference electrode outer filling solution were products of Fisher Scientific (Waltham,

MA). TDMAC was obtained from Polysciences (Warrington, PA). High molecular weight PVC, 2-nitrophenyl octyl ether (NPOE), anhydrous tetrahydrofuran (THF), PQ-2, PQ-6, PQ-10, and PMETAC were purchased from Sigma-Aldrich (St. Louis, MO). NaDNNS was a gift provided by King Industries (Norwalk, CT).

4.2.2 Pulstrode Membrane Preparation

All experiments and membrane preparations were conducted at ambient temperature (22℃). The10 mM phosphate buffer, pH 7.7, with 10 mM NaCl (10 mM PBS) solutions used as diluent or as inner filling solutions, were derived from a 500 mM stock phosphate buffer solution, pH 7.4, containing 500 mM NaCl solution (50x PBS) by dilution. Pulstrode membranes were prepared by solvent casting membranes in the conventional manner as described by Craggs et al.[17] Membrane cocktails contained 10% $TDMA^+$-$DNNS^-$ (neutral lipophilic salt), 30% PVC, and 60% NPOE (w/w). All ingredients were dissolved in approximately 1.4 mL of anhydrous THF and poured into a glass ring (3.55 cm i.d.) placed atop a glass slide (75 x 50 x 1 mm). Solvent evaporation occurred overnight to ensure complete loss of solvent. Small discs (5 mm i.d.) were cut *via* cork borer and integrated into Ostec electrode bodies (Oesch Sensor Technology, Sargans, Switzerland). These electrode bodies contained a Ag/AgCl reference electrode encased in a compartment that can be filled with an appropriate salt solution (inner filling solution). Polymer membrane-based films were integrated into the cap of the electrode body and this cap/membrane served to separate the inner filling solution of the electrode body from the sample phase. The inner filling solutions for each pulstrode sensor were 10 mM PBS.

4.2.3 Three-Electrode System

All experiments were performed using a three-electrode system with a Ag/AgCl double junction external reference electrode, the polyion sensing polymer membrane working electrode, and a counter electrode placed in the sample solution. The external double junction reference electrode contained a gel composed of 3 M KCl within the inner compartment and a 10% KNO_3 solution in the outer compartment. The junction between the inner and outer compartments was a porous ceramic frit while the junction between the outer compartment and sample solution was a pinhole covered by a polytetrafluoroethylene sleeve. The counter electrode was a high surface area Pt wire coil. Prior to direct or indirect detection of any PQ species, all three electrodes were placed in the sample solution and potentials were recorded until a stable baseline potential was attained when the working electrode EMF was measured against the double junction reference electrode. Then, an appropriate galvanostatic, open circuit, and potentiostatic pulse sequence was applied using an AFCBI bipotentiostat system (Pine Instruments, Grove City, PA). Pulse sequences for all experiments consisted of a 1.0 s galvanostatic pulse (anodic or cathodic depending on whether

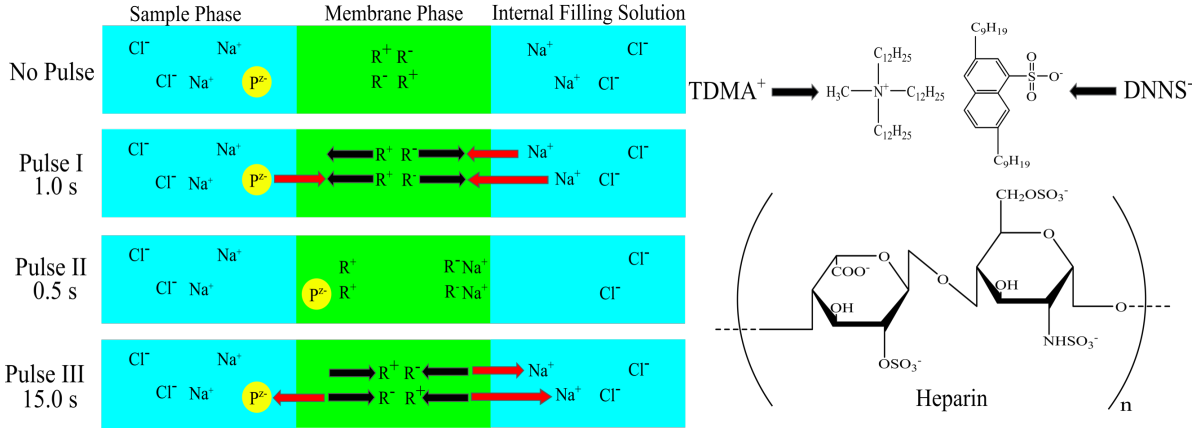

Figure 4.1. Diagram of the pulstrode sequence used for polyion detection. In this diagram, an anodic galvanostatic current pulse was used to detect the polyanion heparin. The neutral lipophilic salt within the membrane phase was $TDMA^+(R^+)$–$DNNS^-(R^-)$. This sequence can also be adapted for polycation detection by switching from an anodic to a cathodic galvanostatic pulse.

polyanion or polycation sensing was required), a 0.5 s zero-current pulse, and a 15.0 s potentiostatic pulse at 0 V (see Figure 4.1).

4.2.4 Direct Detection of PQs

To characterize the total direct ΔEMF response toward each PQ species, the three-electrode pulstrode system was placed into different beakers containing 50 mL each of 10 mM PBS and stable EMF baselines were allowed to develop. Different PQ species at various stock concentrations were injected into each beaker using a dual-syringe infusion/withdrawal pump (Cole-Parmer, Vernon Hills, IL) at a flow rate of 288 μL/min. The applied galvanostatic current pulse for all direct detection experiments was -20 μA. The recorded potentials were measured at the end of the zero-current pulse segment of each cycle. The potentials were sampled as the average value during the last 10% of the zero-current pulse of each cycle. All solutions were stirred continuously *via* magnetic stir bar.

4.2.5 Indirect Detection of PQs

Increasing concentrations of PQs were prepared in individual beakers where there was a total sample volume of 50 mL. The three-electrode pulstrode system was placed into the beakers and a stable baseline output voltage was allowed to develop with the pulstrode operated in the polyanion sensing mode. The dual-syringe infusion/withdrawal pump was used at a flow rate of 288 μL/min to inject the titrant (heparin). The applied current for the galvanostatic pulse was +20 μA for all indirect detection experiments. The open circuit potentials were measured at the end of the zero-current pulse of each cycle. The potentials were sampled as the average value during the

last 10% of the zero-current pulse of each cycle. All solutions were stirred continuously *via* magnetic stir bar.

4.2.6 PQ-6 Quantification in Swimming Pool Water

Samples of water from a recreational swimming pool in Ann Arbor, MI were collected in a glass container and stored at room temperature. Working solutions of increasing concentrations of PQ-6 were contrived by doping individual pool samples with increasing concentrations of PQ-6 (100, 200, 300, and 400 μg/mL). These samples were individually diluted 1:5 in a 200 mL volumetric flask with deionized water and 2 mL of a 500 mM phosphate buffer, pH 7.4, with 10 mM NaCl. The resultant sample for each concentration of PQ-6 therefore contained a background electrolyte of at least 5 mM phosphate buffer, pH approximately 7.3, with 0.1 mM NaCl, in addition to what is present in the pool sample originally. A blank sample containing 0 μg/mL PQ-6 was also prepared in the same fashion as the working solutions of increasing concentrations of PQ-6 contrived *via* PQ-6 addition. The blank was diluted 1:5 in the same fashion as the solutions containing doped PQ-6.

Aliquots of 50 mL each were used for titrations of each concentration of PQ-6; the final concentrations of PQ-6 were 0, 20, 40, 60, and 80 μg/mL after the 1:5 dilution/buffer addition. The three-electrode pulstrode system was placed into the beakers in the same fashion as indirect and direct experiments. A stable baseline was recorded in polyanion sensing mode prior to any experiment. The dual-syringe infusion/withdrawal pump was used at a flow rate of 291 or 201 μL/min to inject the titrant (heparin). The galvanostatic pulse for all pool sample experiments was +20 μA. The open circuit potentials were measured at the end of the zero-current pulse of each

cycle. The potentials were sampled as the average value during the last 10% of the zero-current pulse of each cycle. All solutions were stirred continuously *via* magnetic stir bar.

4.2.7 FIA System

Three samples of increasing concentrations of PQ-6 (30, 60, and 90 µg/mL) were prepared in volumetric flasks by diluting a working solution of 80 mg/mL PQ-6. The working solution was derived from a stock solution of 20 wt. % PQ-6 in water purchased from Sigma-Aldrich and diluted with 10 mM PBS. Before each solution of PQ-6 was fully diluted to mark, a volume of concentrated heparin was added to create a final heparin concentration of 150 µg/mL in each flask of PQ-6. One additional solution of solely 150 µg/mL heparin was prepared in the same fashion. Each of these solutions were sequentially injected into the sample loop of the FIA system in triplicate for detection of the PQ-6 species present.

Figure 4.2 shows a schematic of the FIA system which was used for the semi-automated detection of the PQ species. This system was assembled using TFE Teflon tubing (0.8 mm i.d. x 1.58 mm o.d.) (Sigma-Aldrich, St. Louis, MO) throughout. Both carrier stream and injection plugs were controlled using a 6-port manual rotary injection valve (VICI, Houston, TX). A peristaltic pump (MINIPULS 3, Gilson, Middleton, WI) was used to introduce the carrier stream into the system (10 mM PBS). The volume of the sample loop and the flow rate of the FIA system were 250 µL and 915 µL/min, respectively. Lastly, the applied galvanostatic current was +20 µA for all experiments. The open circuit potentials were measured at the end of the zero-current pulse of each cycle. The potentials were sampled as the average value during the last 10% of the zero-current pulse of each cycle.

4.3 Results and Discussions

4.3.1 Direct Detection of Four PQ Species

Each polycation in this current work can induce a super-Nernstian response *via* non-equilibrium partitioning of the polycation across the sample-membrane interface when using a single-use polyion sensor for detection.[8] However, because conventional polyion sensor response is considered irreversible when using these sensors, it is important to demonstrate that PQs can illicit similar behavior using the pulstrode system. Figures 4.3a-b show the measured total ΔEMF observed for each PQ in 10 mM PBS. Each PQ species exhibits different total ΔEMF response curves that are indicative of their chemical properties (e.g., molecular weight, diffusion coefficient in the PVC membrane, charge density, etc.). Interestingly, PQ-10 exhibits a relatively low total

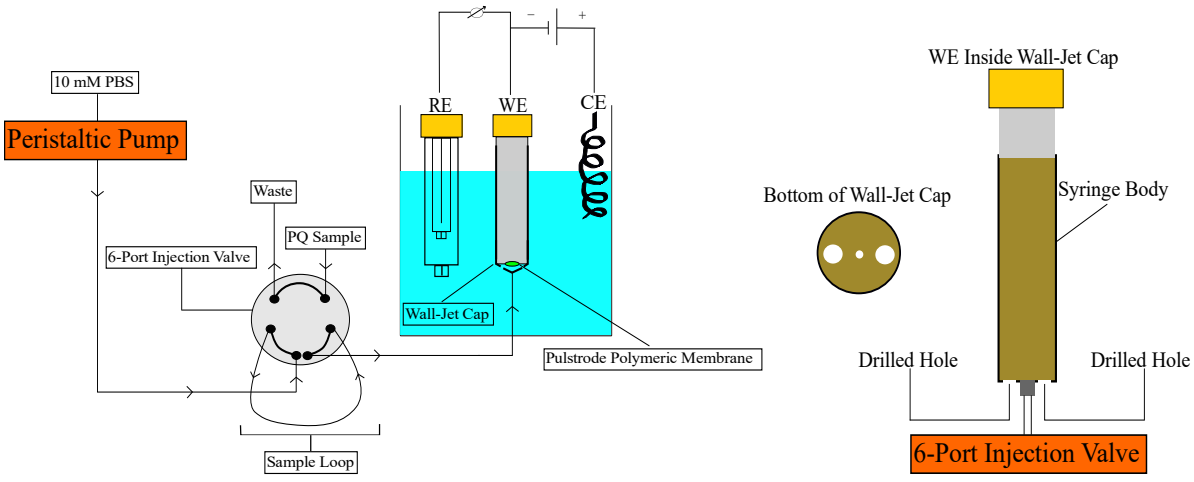

Figure 4.2. Schematic of the FIA system (left) used for indirect PQ detection/quantification and a schematic of the working electrode placed inside a wall-jet cap (right). A carrier stream of 10 mM PBS was used in conjunction with PQ/heparin samples that were manually injected using the sample loop of the 6-port injection valve.

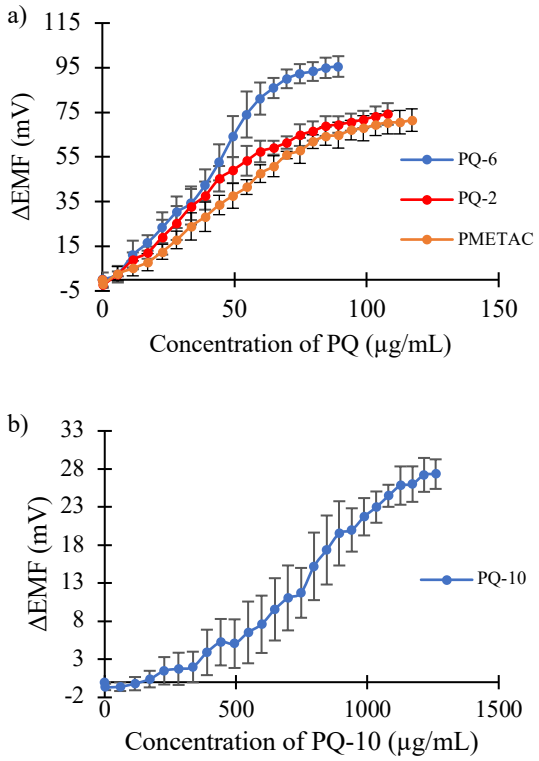

Figure 4.3. Total ΔEMF of four different PQ species in 10 mM PBS. The data points in each curve respresent the average ΔEMF of three separate dose-response experiments as followed by a pulstrode polyion sensor.

ΔEMF response when compared to PQ-2, PQ-6, and PMETAC and much higher concentrations are needed to observe this response. This is similar to the results found previously when using single-use polyion sensors,[8] where the large difference between the PQ-10 total ΔEMF response and the remaining PQs can be attributed to the relatively low charge density of the PQ-10 species.[18] Low charge density decreases the thermodynamics for extraction of the PQ species into the PVC membrane that is polarized (*via* the galvanostatic pulse) to have excess of the $DNNS^-$ species at the outer sample side surface of the membrane.

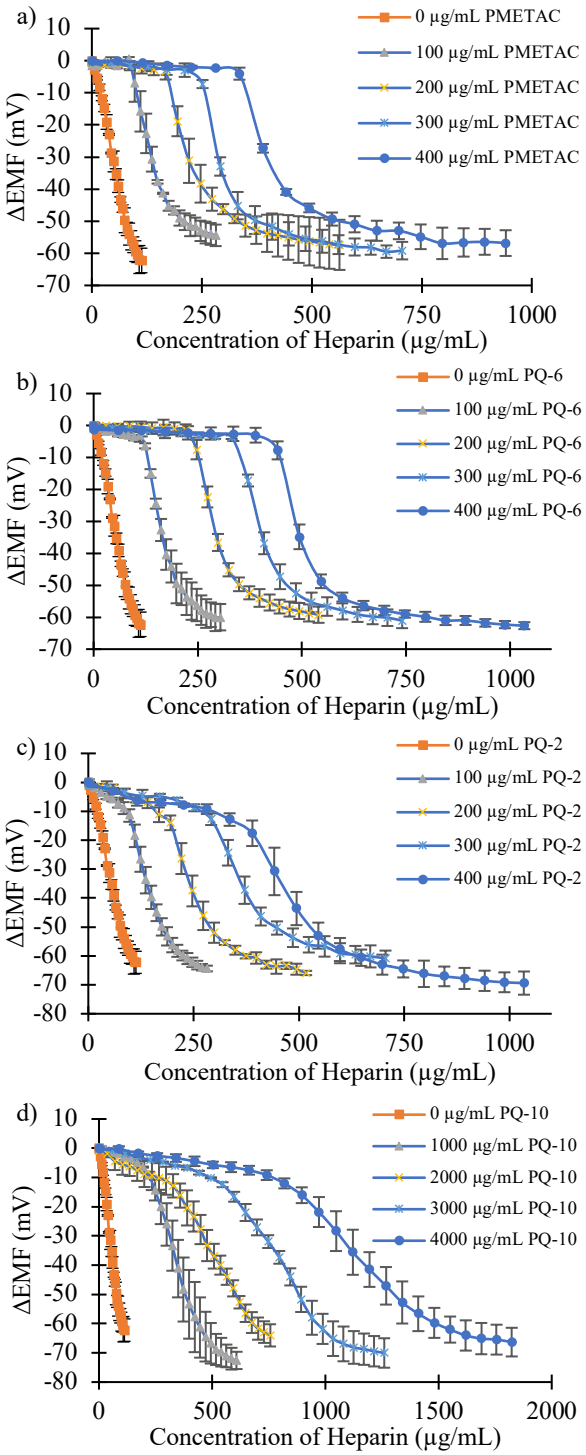

Figure 4.4. Potentiometric titrations of (a) PMETAC, (b) PQ-6, (c) PQ-2, and (d) PQ-10. All data points represent the average ΔEMF value for each concentration of heparin as calculated from three separate titrations using the pulstrode system.

4.3.2 Indirect Detection of PQs

Given the wide variety of PQ species available that differ in chemical structure, charge density, diffusion behavior, etc. direct detection may pose significant concerns for reproducible detection and quantification. For instance, if the charge density of the species is significantly low (as is the case for PQ-10) the total ΔEMF will be too small to be considered analytically useful. To circumvent this limitation PQ concentrations can be more reliably quantified *via* an indirect detection method. Figures 4.4a-d show the potentiometric titrations of each PQ species as followed by the pulstrode system in the polyanion sensing mode using heparin (a polyanion) as the titrant. Each titration curve of increasing concentrations of a particular PQ is shifted to the right as it requires more heparin to interact and bind tightly to the given PQ species in the 10 mM PBS background electrolyte. In addition, the total ΔEMF of each curve regardless of the PQ species being titrated maintains a relatively stable value of approximately -70 mV, representing the maximum polyanion response to excess heparin using the pulstrode.

Further, the first derivative plot of each of these curves can be calculated, thus allowing an equivalence point for each curve to be determined. Figures 4.5a-b show the equivalence points of each titration curve for their respective PQ species. For each PQ species, there is a linear response that can be used for quantification or to determine specific chemical characteristics including stoichiometry. For instance, because PQ-6 has the highest slope value of the four PQs it can be inferred that PQ-6 has the highest charge density of these species. This finding agrees with the data presented previously using single-use polyion sensors to follow the titrations.[8] The order of the slope values for the remaining PQs are also in agreement with this prior study. The limits of detection (3σ) for PQ-2, PQ-6, PQ-10, and PMETAC for titrations using the pulstrode system are 8.0, 7.9, 35.5, and 10.3 μg/mL, respectively. Poorer detection limits for PQ-10 is anticipated based

on the lower charge density of this polycation species. Lastly, it is important to note that the concentrations of each solution of PQ-10 is significantly higher than the concentrations of the other PQ species. This was done to demonstrate a detectable concentration range that would be appropriate for PQ-10 concentrations in more complex matrices (i.e., shampoo samples). PQ-10 is known to be used as a conditioning agent in shampoo formulations and is usually found in concentrations ranging from 0.01% - 10% by mass.[19–21]

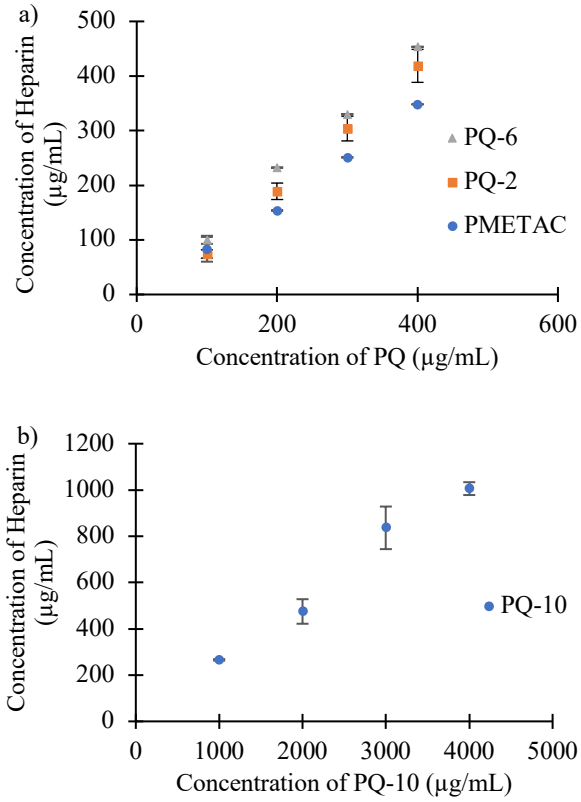

Figure 4.5. Calibration curves derived from the equivalence points calculated from the first derivative plots of the titration curves found in Figure 4.4.

4.3.3 PQ-6 Quantification in Pool Samples

As PQ-6 can be used in pool and spa environments designated for recreational use,[15,16] it is important to demonstrate the ability of this sensor-based titration method to be applied to the

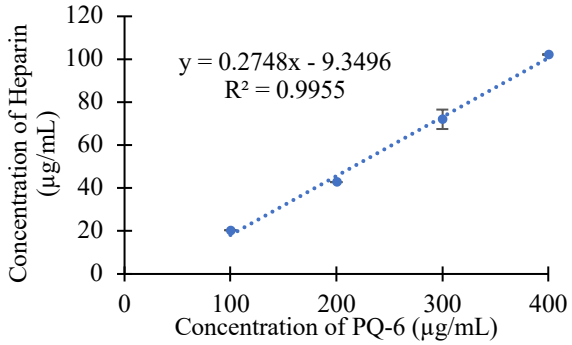

Figure 4.6. Calibration curve derived from the equivalence points calculated from the first derivative plots of titrations performed using diluted pool samples spiked with PQ-6. (note: concentrations on the x-axis are concentrations of PQ-6 within pool samples before dilution measurements as indicated in the text).

detection/quantification of PQ-6 in these types of matrices. Such samples also contain a number of other agents, including chlorine, chloramines, hydrogen peroxide, algaecides, etc.[15,22] Figure 4.6 shows the calibration curve for PQ-6 at relevant concentrations that can be found in swimming pools when plotting the equivalence points of the heparin titrations vs. PQ-6 levels. The calibration curve exhibits the expected linear response to the excess polyanion titrant, heparin. In addition, two samples containing 150 and 250 µg/mL PQ-6 were contrived in fresh pool water and diluted in the same fashion as the calibration standards. Each sample was titrated with heparin in triplicate. The blank sample equivalence point value was subtracted from the average equivalence points of each sample (n=3) and these values were used to calculate the concentration of PQ-6 via the calibration curve generated from the pool water calibration standards. The average calculated PQ-6 concentrations in each sample were found to be 154.7 ± 0 and 249.8 ± 11.6 µg/mL for the 150 and 250 µg/mL (nominal values) samples, respectively. The percent difference of these values when compared to the nominal values were 3.11% and 0.09% for the 150 and 250 µg/mL pool samples, respectively.

4.3.4 PQ Detection and Quantification via FIA System

As titrations can be cumbersome and time consuming, it is logical to pursue a more automated detection method for PQ analysis. Toward this goal, an FIA system was targeted as an effective means by which PQs can be quantified more quickly and efficiently. Figure 4.7 shows the results for injections containing 150 µg/mL heparin in 10 mM PBS each using a carrier stream of 10 mM PBS. These injections were made using a six-port injection valve and allowed to flow across the pulstrode membrane outer surface using a wall-jet configuration. The resulting negative EMF response recorded by the pulstrode system can be attributed to the free heparin in solution being able to partition into the membrane and interact with the oppositely charged $TDMA^+$ species that migrates to the sample- membrane interface due to the anodic galvanostatic current pulse.

Figure 4.8a shows the responses observed for a series of injections using four different mixtures of PQ-6 and heparin in which heparin was kept at a constant concentration while the PQ-6 concentration was varied. Each of these solutions was injected into a 250 µL sample loop and subsequently allowed to flow over the pulstrode membrane. The heparin in the solution was able

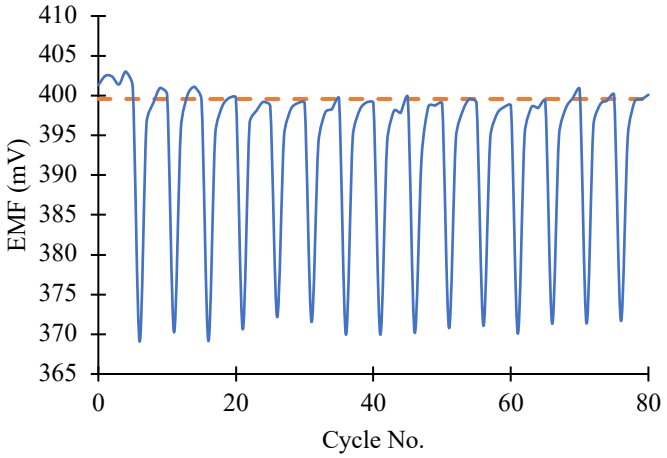

Figure 4.7. Injections of 150 µg/mL heparin in 10 mM PBS. The average ΔEMF for the peaks is -28.94 mV ± 0.91 mV. The dotted line represents the average baseline potential value for the point immediately prior to each of the 15 peak vertices.

to interact with the $TDMA^+$ species that was located at the sample side of the plasticized PVC membrane as a result of the anodic galvanostatic current pulse applied to the working electrode for 1.0 s, thus generating a negative potential on the dynamic potentiometric response plot. The depth of each trough corresponds to the amount of free heparin remaining in the sample, which is inversely related to the amount of PQ-6 in the original sample (i.e., the more PQ species, the less free heparin). The resulting calibration curve shown in Figure 4.8b displays the expected linear response relating the ΔEMF calculated from the dynamic potentiometric response curve in Figure 9a to the concentration of PQ-6 in each calibration standard.

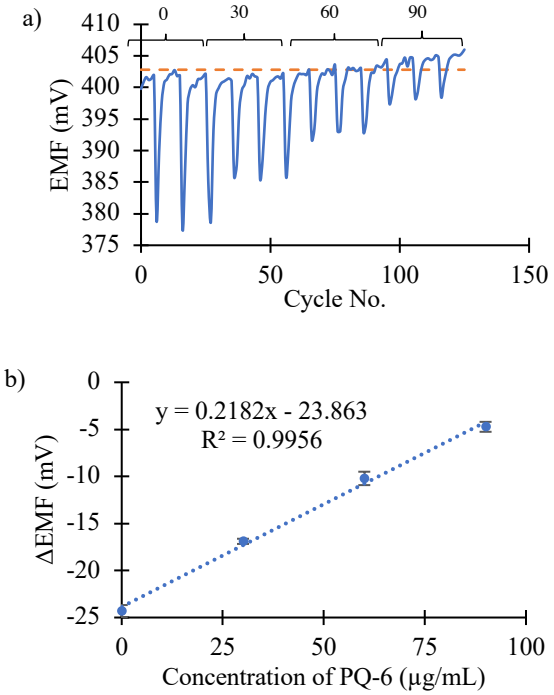

Figure 4.8. (a) Dynamic potentiometric response to excess heparin in each mixture of 150 µg/mL heparin and increasing concentrations of PQ-6. The pulstrode system is in polyanion detection mode and the solid line shows the negative peaks generated from excess heparin. The dotted line represents the average baseline potential value for the points occurring immediately prior to each of the 12 peak vertices. The numbers above each bracket represent the concentration of PQ-6 (µg/mL) present in each injected solution also containing 150 µg/mL heparin. (b) Calibration curve derived from the dynamic potentiometric responses shown in (a).

4.4 Conclusions

We have demonstrated that fully reversible polyion-sensitive polymer membrane-based pulstrodes can be used to detect and characterize various PQ species by observing their EMF responses. As these compounds are difficult to measure by other means, this new approach provides a robust and reliable method to quantify these polycations. Both direct and indirect methods can be used; however, the indirect detection method based on titrations using the pulstrode to sense excesses of the polyanion titrant (heparin) is the preferred and more universal method given the diverse nature of the large number of PQ species commercially available. This can be attributed to some PQ species (e.g., PQ-10) exhibiting relatively small total ΔEMF responses when using the direct polycation detection mode.

Further, we have shown that this method of PQ detection can be performed in real sample matrices such as recreational swimming pool water with good accuracy. In addition, we have obtained preliminary data demonstrating that this method can be adapted to a FIA system in which mixtures of varying PQ levels and a fixed heparin concentration can be injected to yield negative EMF response peaks that are inversely proportional to the level of PQ species present. While demonstrated here for PQ-6 detection, this FIA method is readily adaptable to detect many other PQ species.

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CHAPTER 5

Detection and Quantification of Polyquaterniums (PQs) *via* Polyion-Sensitive Ion-Selective Optodes Inkjet Printed on Cellulose Paper

Ferguson, S.A.; Wang, X.; Mahoney, M.; Meyerhoff, M.E. *Anal Sci.* **2018**; 34 (1): 45-50 *

5.1 Introduction

Ionic polymers (polyions) are important macromolecules that contain multiple charges throughout their structures and have a diverse number of applications. Polymeric quaternary ammonium salts (polyquaterniums (PQs)) are examples of such polyions and exhibit multiple positive charges (i.e., polycations). These positive charges are derived from quaternary ammonium functional groups located along the polymer chain and the charge of these polycationic species is independent of the solution pH.[1] PQs are considered "strong" polycations as opposed to "weak" polycations, which are polymer species that acquire positive charge only in acidic environments.[2] PQs are found to be useful as conditioning agents in shampoos,[3–5] additives in fabric softener compositions[6,7] and lotions,[8,9] zebra mussel biocides,[10] antimicrobials/preservatives in contact lens solutions,[11,12] and as algaecides in spas and swimming pools.[13]

* All experiments, data analysis, and figure construction for this chapter were performed by Stephen A. Ferguson. Xuewei Wang fabricated the polyion-sensitive ion-selective optodes (ISOs). Mark E. Meyerhoff was the principal investigator for the project. P&G and the MBSTP at the U-M (NIH grant number T32 EB005582) funded the entirety of the study.

As PQs have grown in their use/applications, it is becoming more important to develop methods and devices capable of quantifying and characterizing these polymeric species. It is well known that traditional methods for polyion detection can be challenging, as these molecules have high molecular weights and do not absorb light at wavelengths >260 nm.[14] In addition, traditional electrochemical methods of detection are often not suitable since PQs do not possess redox active domains for analysis *via* conventional cyclic voltammetry[14,15] or related amperometric/voltammetric techniques.

To circumvent this challenge, it was recently demonstrated that polyion-sensitive ion-selective electrodes (ISEs) can be used to quantify and characterize PQ species in solution.[16–18] These studies showed that both single-use and fully reversible (pulstrode) polyion sensors can be employed to obtain significant potentiometric responses toward PQ-6, PQ-2, PQ-10, and poly(2-methacryloxyethyltrimethylammonium) chloride (PMETAC) (see Figure 1.6 for structures) in a simple background electrolyte[16,18] and more complex matrices such as partially treated drinking water[18] and recreational swimming pool water.[17]

Despite the success that both single-use and pulstrode-type polyion sensors have shown with respect to PQ detection/quantification, these sensors still require macroelectrodes and specialized equipment to convert the analog potentiometric response signal to a digital signal, making in-field applications more challenging. Earlier efforts have demonstrated that optical sensing films for the direct detection of polycations (e.g., protamine) and the indirect detection of polyanions (e.g., heparin) can be created by incorporating a lipophilic fluorescein ester into thin polymeric films coated on the wells of microtiter plates.[19] However, such optical polyion sensors exhibit rather long response times (e.g., 15 min.) owing to the need for extraction of the polyions into the bulk organic polymeric phase.

Over the last several years paper-based devices have gained popularity as they allow for more streamlined, facile, and simplistic methods of detection for a variety of analytes in different matrices. These devices have been used to detect aluminum, iron, urinary creatinine, Hg^{2+}, Co^{2+}, and Zn^{2+}, among others.[20–23] In accordance with this observed trend, ion-exchanger-based ion-selective optodes (ISOs) printed directly on the surface of cellulose paper (without need for plasticizer or additional polymer) have been shown to be capable of detecting/quantifying polyions using colorimetry *via* smartphone analysis, with response times of 5 min.[24] In this very recent work, polyions were detected using an inkjet-printed ISO containing dinonylnaphthalenesulfonic acid (H^+DNNS^-) and chromoionophore I. This sensor system relies on ionic interactions between the adsorbed ion-exchanger ($DNNS^-$) and protamine (a polycation). The subsequent proton release from chromoionophore I facilitates a measurable change in hue (blue to purple).[24]

Herein, we describe the application of this new colorimetric polycation-sensitive ISO technology for the quantification and characterization of PQ-6, PQ-2, PQ-10, and PMETAC. We further demonstrate how the level of free, detectable PQ can be altered in the presence of different levels of a polyanion (e.g., dextran sulfate (DS)) and detected *via* polycation-sensitive ISOs, thereby providing an indirect method to quantify the concentration of a given polyanion in solution. Lastly, we show that PQ-6 can be quantified in recreational swimming pool samples at ppm levels in addition to exploring the quantification of PQ-6 in water samples by drying a buffer directly onto a polycation-sensitive ISO membrane, creating a dried buffering system on the surface of the sensor for local pH control of the applied liquid sample.

5.2 Experimental Section

5.2.1 Reagents and Chemicals

Sodium chloride and Whatman™ qualitative filter paper (grade 5; diameter: 18 cm; thickness: 200 μm) were purchased from Fisher Scientific (Waltham, MA). Sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate were products of Amresco, Inc. (Solon, OH). PQ-6, PQ-2, PQ-10, PMETAC, and chromoionophore I were purchased from Sigma-Aldrich (St. Louis, MO). HDNNS was a gift provided by King Industries (Norwalk, CT).

5.2.2 Preparation of Parent Polycation-Sensitive ISOs

Parent polycation-sensitive ISOs were batch fabricated by dissolving 0.92 mg HDNNS and 1.16 mg chromoionophore I in 580 μL cyclohexanone. This cocktail was decanted into a Dimatix Materials Cartridge (DMC-11610, 10 pL drop size) and directly printed onto the filter paper using a Dimatix MP-2831 Inkjet Printer. The cartridge nozzle and the platen were at room temperature while drop spacing was 25 μm. The number of layers used for printing each parent polycation sensing layer was one for all batches; maximum jetting frequency was 5 kHz.

5.2.3 Direct PQ Detection in Buffer via Polycation-Sensitive ISOs

The 10 mM phosphate buffer, pH 7.6, with 10 mM NaCl (10 mM PBS) used as a diluent for all experiments was prepared by diluting a 100 mM phosphate buffer, pH 7.4, containing 100 mM NaCl solution unless otherwise noted. Increasing concentrations of PQ-2 and PQ-6 were contrived by serially diluting 62 wt% in water and 20 wt% in water solutions, respectively, of these polyions with 10 mM PBS. The resulting sample concentrations were 1, 10, 25, 50, 75, 100, 500, and 1000 μg/mL PQ-2 or PQ-6. PQ-10 and PMETAC solutions were prepared by dissolving the

salt of each species in 10 mM PBS to make stock solutions of each species. These stock solutions were then serially diluted to make PQ samples containing 1, 10, 25, 50, 75, 100, 500, and 1000 μg/mL of PQ-10 or PMETAC.

To prepare the polyion-sensitive optodes, small discs (0.60 cm diameter) were cut from the parent sensing paper (4.95 cm diameter) using a standard hole punch (Sparco™ Brand) and mounted on the back of 96-well microtiter plates by pressing each polycation-sensitive ISO into a square piece of parafilm®. Thirty microliter samples of each concentration of a single PQ species were aliquoted directly onto each sensing layer and allowed to incubate at room temperature on the benchtop for 5 min. Immediately prior to the end of the 5-min. period, each microtiter plate was placed in a homemade dark box. Three pictures of each sample-containing sensor were taken in succession and hue values were extracted from each photograph *via* the iPhone application "Color Mate."[25] Further, the optical signal of each concentration of solely PQ-6 was also measured using two additional polycation-sensitive ISOs to assess sensor-to-sensor variability. Photographs of each additional polycation-sensitive ISO used for PQ-6 detection were taken in triplicate.

5.2.4 Indirect DS Quantification via Polyion Precipitation Using Polycation-Sensitive ISOs

Samples of 50 μg/mL PQ-6 containing increasing concentrations of DS were made in 10 mL volumetric flasks. The final concentrations of DS in each flask were 22, 29, 36, and 43 μg/mL. Small sensor discs were prepared for sample testing and sample photographs were taken, both in the same fashion as direct PQ-6 detection experiments.

5.2.5 Quantification of PQ-6 in Swimming Pool Samples

Recreational swimming pool samples were collected in glass containers and stored at room temperature on a benchtop. Increasing concentrations of PQ-6 were added to 10 mL volumetric flasks using working stock solutions of PQ-6 in distilled water. PQ-6 was added in each flask to create final concentrations of 1, 10, 25, 50, 75, 100, 500, and 1000 µg/mL PQ-6. In addition to the PQ-6 additions, 800 µL of a 500 mM phosphate buffer, pH 7.4, containing 10 mM NaCl, was added to each flask. The resulting final buffer concentration was 40 mM phosphate, pH 7.5, with 0.8 mM NaCl. Each mixture was then diluted to mark with untreated pool water. Small sensor discs were prepared for sample testing and sample photographs were taken, both in the same fashion as direct detection experiments described above for PQ-2, PQ-10, and PMETAC.

5.2.6 Quantification of PQ-6 in Swimming Pool Samples Using Optodes Soaked in Buffer

Parent polycation-sensitive ISOs (4.95 cm diameter) were batch fabricated and subsequently soaked in a 100 mM phosphate buffer, pH 7.5, with 2 mM NaCl for no more than 1 min. in a petri dish. The parent sensor films were then removed from the buffer solution and excess buffer solution was wicked off the membrane using fresh filter paper. The buffer-soaked parent sensors were then placed in a 37℃ oven and allowed to dry for 30 min. Samples of increasing concentrations of PQ-6 were prepared in distilled water using 10 mL volumetric flasks to make 1, 10, 25, 50, 75, 100, 500, and 1000 µg/mL PQ-6 sample solutions. Pool samples containing identical concentrations of PQ-6 were also prepared in 10 mL volumetric flasks by doping an untreated pool sample with concentrations of PQ-6. Small sensor discs were prepared for sample testing and sample photographs were taken using the same method described for direct detection experiments for PQ-2, PQ-10, and PMETAC.

5.3 Results and Discussions

5.3.1 Direct PQ Detection

As there are many PQ species that can differ in charge density, molecular weight, molecular structure, etc., it is important to develop a universal method by which PQs can be characterized and quantified. Polyion-sensitive ISOs represent a facile alternative to polyion-sensitive ISEs. To demonstrate their application to PQ analysis, PQ-6, PQ-2, PQ-10, and PMETAC were used as model PQ species for polycation-sensitive ISO detection. Figure 5.1 shows photos of a series of polycation-sensitive ISOs in the presence of increasing concentrations of PQ-6. As the concentration of PQ-6 increases, the final hue of each sensor changes progressively from blue to purple. This demonstrates the ability of such polycation-sensitive ISOs to be used for PQ-6 detection/quantification. Figures 5.2a-d show the polycation-sensitive ISO response (hue) to increasing concentrations of each PQ species. These responses exhibit the expected sigmoidal response curve, the magnitude of which is different for each PQ species. It is important to note that PQ-10 exhibits the smallest change in hue once an equilibrium response is obtained. This can be attributed to the very low charge density of the PQ-10 species relative to the other PQs tested. Conversely, PQ-6 shows the highest change in hue when compared to the remaining PQs which can be attributed to its relatively high charge density.[26,27] The low charge density of PQ-10 makes

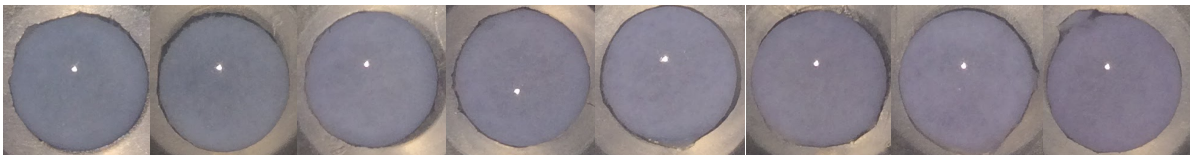

Figure 5.1. Photographs taken of each polycation-sensitive ISO containing increasing concentrations of PQ-6. Each photograph was taken after five minutes of incubation on the benchtop.

it favorable for use in cosmetic applications (e.g., shampoos, conditioners, etc.).[26,28] The relatively high charge density of PQ-6 makes this species a preferable flocculating agent in water treatment processes.[29]

It is important to determine the variability of hue values from sensor to sensor. To accomplish this, we used PQ-6 as a model PQ species to show the ability of these polycation-sensitive ISOs to generate a stable and reproducible hue value per concentration level between

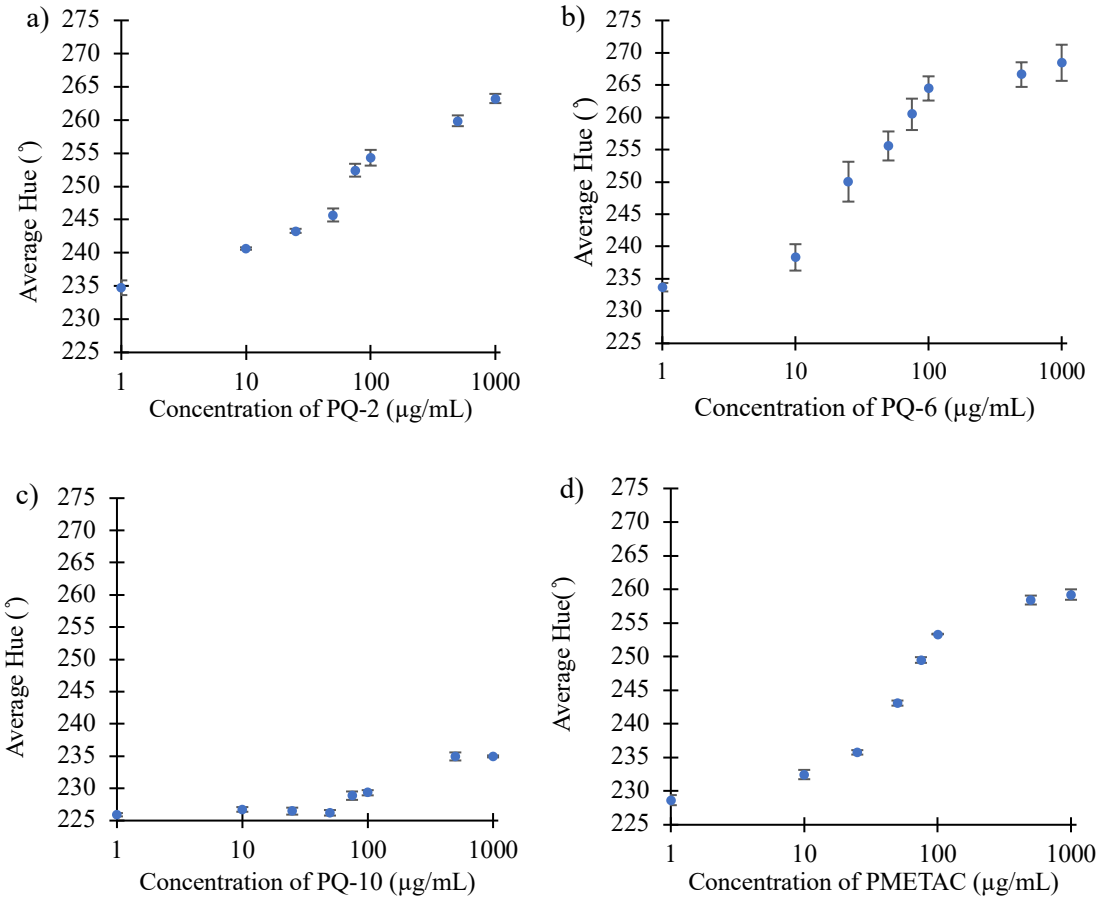

Figure 5.2. Direct response curves for a) PQ-2, b) PQ-6, c) PQ-10, and d) PMETAC. Data points for PQ-2, PQ-10, and PMETAC are derived from hue values extracted from three different photographs of the same sensor taken five minutes after initial addition of the sample to the sensor. Data points for PQ-6 are derived from triplicate photographs of three different polycation-sensitive ISOs averaged together resulting in three averaged hue values for each concentration level. These three values were then averaged to generate the data points with error bars shown in Figure 2b. For all PQs displayed the x-axis is plotted logarithmically (base 10).

sensors. Each data point in Figure 5.2b for PQ-6 is derived from an average of three polycation-sensitive ISO hue values which were calculated by averaging three hue values extracted from separate pictures taken of the same polycation-sensitive ISO site (performed in triplicate). With the exception of the data for PQ-6, triplicate photographs of the same sensor were taken for the remaining PQ samples and standard deviations were calculated from these three measurements. The latter method of hue value determination through photographic analysis *via* "Color Mate" was chosen for all PQ analyses, other than PQ-6, to reduce the rate of sensor consumption and to reduce required testing times for each response curve. Reduced testing times would be ideal for in-field analyses. There is a slight difference in the magnitude of the error bars between the method of hue analysis performed for PQ-6 vs. the remaining PQ species (i.e., PQ-6 shows slightly higher variability). However, both methods show adequate and reproducible response and the most appropriate method can be chosen depending on the application.

5.3.2 Indirect DS Quantification

These new polycation-sensitive ISOs are also capable of indirectly quantitating polyanions. This involves mixing a fixed concentration of a PQ species with a sample containing a polyanion (e.g., DS) which can bind strongly with a PQ species. By keeping the concentration of the particular polycation (e.g., PQ-6) constant in all sample vials while varying the concentration of DS added to each sample, the response to free (unbound) levels of the PQ species can be sensed by polycation-sensitive ISOs *via* hue changes. Figure 5.3a shows the complete dynamic range of indirect DS detection experiments made for a series of samples containing a fixed concentration of 50 μg/mL PQ-6 along with varying concentrations of DS, Figure 5.3b shows a calibration curve

of these same indirect DS detection experiments based on the linear portion of curve shown in Figure 5.3a. Polycation-sensitive ISOs were used in conjunction with "Color Mate" to measure the hue of each sensor after a 5-min. incubation period. The calibration curve shows good linearity

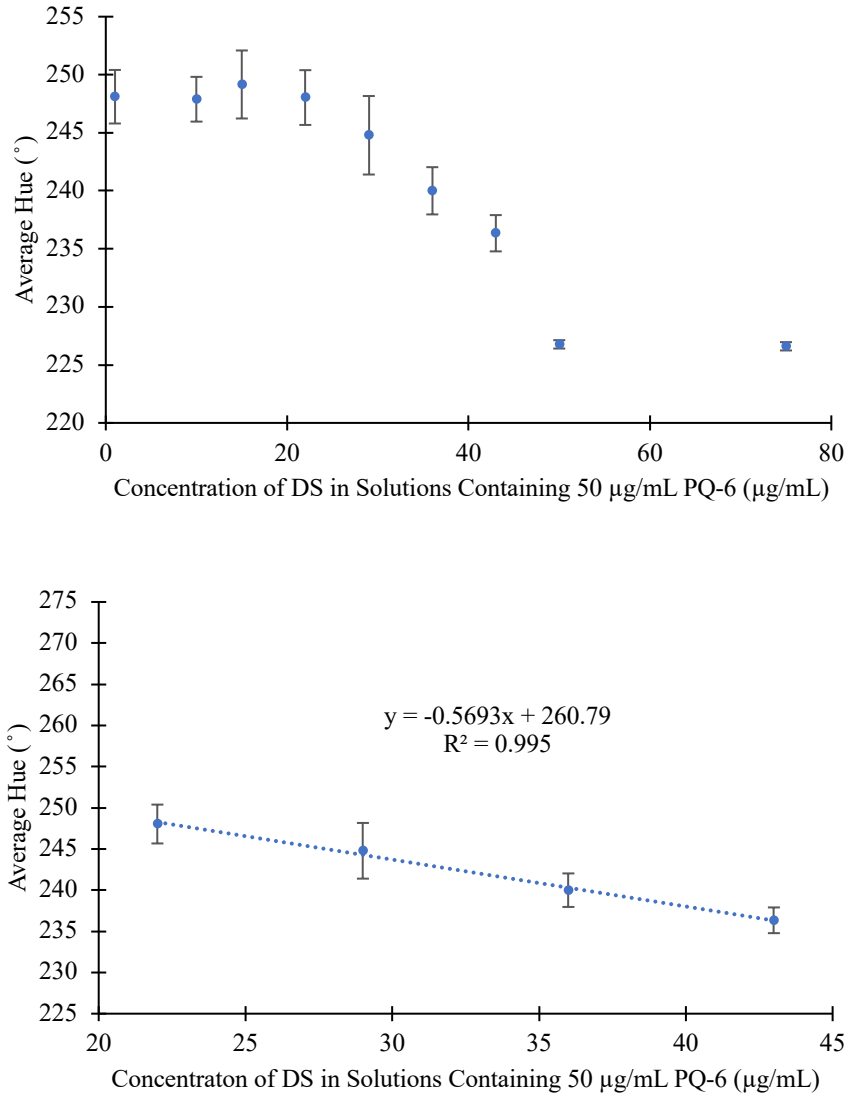

Figure 5.3. a) Dynamic range for the indirect detection of DS using a polycation-sensitive ISO. Each data point is generated from triplicate photographs of three different polycation-sensitive ISOs averaged together resulting in three average hue values for each concentration level. These three values were then averaged to generate the data points with error bars. b) Calibration curve derived from mixtures of 50 µg/mL PQ-6 and increasing concentrations of DS. Each data point is generated from triplicate photographs of three different polycation-sensitive ISOs averaged together resulting in three averaged hue values for each concentration level. These three values were then averaged to generate the data points with error bars.

toward the DS polyanion and demonstrates that polycation-sensitive ISOs can likely be used to indirectly quantitate unknown concentrations of polyanionic species, provided that the polyanion binds the PQ indicator species with high affinity. It should be noted that the variability of the sensor-to-sensor response is similar to the variability shown in the sigmoidal direct response curve of PQ-6. While this variability is slightly higher than that of the variability shown for the remaining PQs the sensors generate adequate responses for each DS/PQ-6 mixture.

5.3.3 Quantification of PQ-6 in Swimming Pool Samples

Since the addition of PQ-6 to recreational swimming pools to improve the feel of hair and skin after swimmers cease contact with the water has been explored,[30] in addition to its use as a potential algaecide,[13] one potential application of the new polycation-sensitive ISOs would be the monitoring of PQ-6 levels in such samples. Figure 5.4 shows the direct response to increasing levels of PQ-6 in swimming pool water collected from a recreational swimming pool in Ann Arbor,

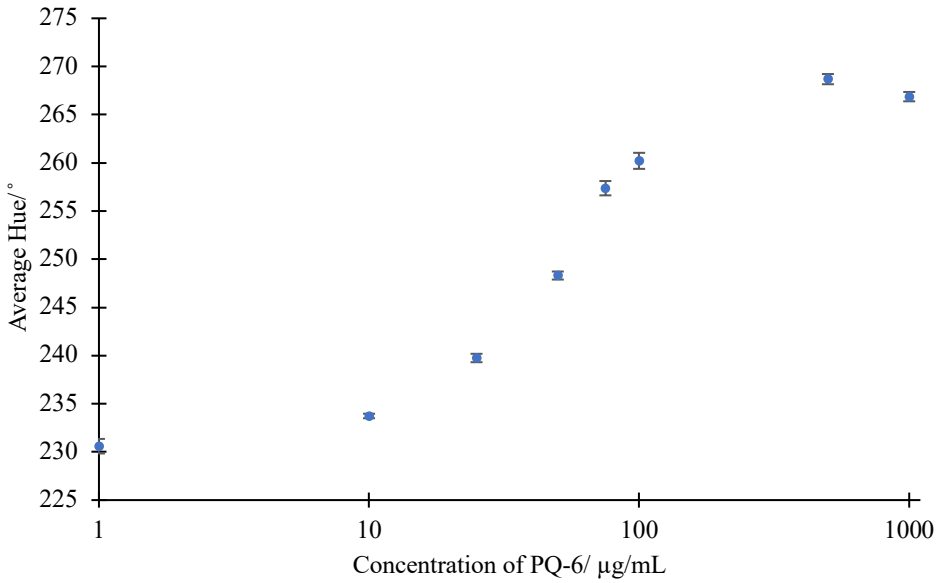

Figure 5.4. PQ-6 in pool water response curve. Each data point is derived from hue values extracted from three different photographs of the same sensor taken five minutes after initial addition of the sample to the sensor. The x-axis is plotted logarithmically (base 10).

MI. The response curve shows the typical sigmoidal shape of a conventional PQ direct detection hue change response curve. In addition, the response curve for the pool samples spiked with PQ-6 is quite similar to the direct response curve of PQ-6 obtained in 10 mM PBS (see Fig. 5.2b). The difference in the two curves might be attributed to the differences in buffer compositions, where the direct response was performed in 10 mM PBS while the swimming pool samples were performed in 40 mM phosphate, pH 7.5, with 0.8 mM NaCl present.

5.3.4 Quantification of PQ-6 Using Sensors Soaked in Buffer

The control of pH is highly important in polycation-sensitive ISOs since the hue change observed for each sensor is facilitated by a proton equilibrium with chromoionophore I adsorbed to the cellulose fibers. As such, samples often need to be tested in buffered solutions to ensure that the proton activity in contact with the optical sensing film deposited on the cellulose paper is relatively constant. To this end, buffer would need to be added as a diluent for all samples; this

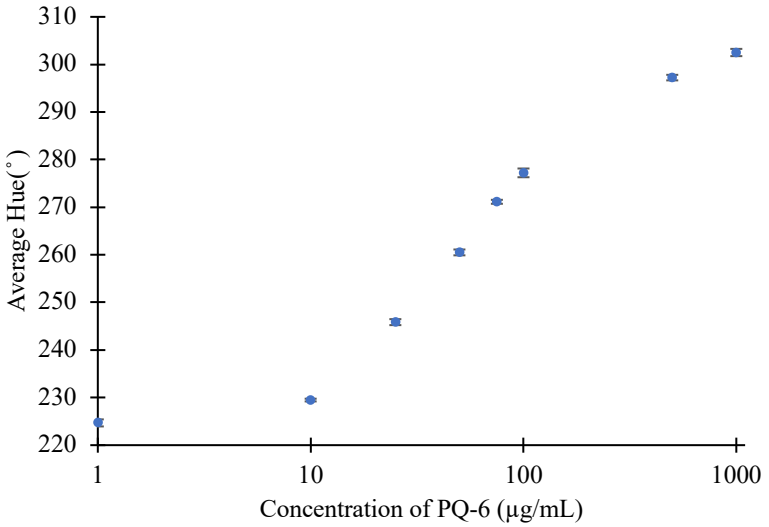

Figure 5.5. Direct response curve of PQ-6 dissolved in distilled water as detected using polycation-sensitive ISOs soaked in 100 mM phosphate buffer, pH 7.5, with 2 mM NaCl. Each data point is derived from hue values extracted from three different photographs of the same sensor taken five minutes after initial addition of the sample to the sensor. The x-axis is plotted logarithmically (base 10).

requires sample pretreatment. It is therefore important to develop a faster more streamlined approach in which the sample can be applied directly to the polycation-sensitive ISOs. This can be accomplished by using polycation-sensitive ISOs soaked in a buffer solution and subsequently dried.[31]

Figure 5.5 shows the response curve toward PQ-6 dissolved in distilled water after it was added at increasing concentrations to polycation-sensitive ISOs prepared with a dry buffer layer and allowed to incubate at room temperature for 5 min. The PQ-6 was dissolved in distilled water for these experiments so that the sample would be solely buffered by the dried buffer system on each sensor site. The response curve toward PQ-6 using the dried buffering system shows the expected sigmoidal curve for a PQ species. Further, Figure 5.6 shows the response curve toward PQ-6 in untreated pool water using the sensors prepared with pre-dried buffer. These data show the same sigmoidal behavior as the PQ-6 samples dissolved in distilled water. This suggests that the dry buffer is able to control the local pH of the pool water samples at pH 7.5. In addition, the

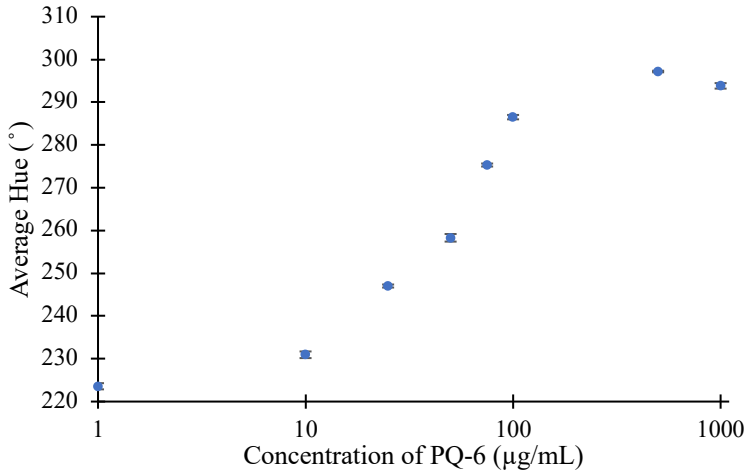

Figure 5.6. Direct response curve of PQ-6 dissolved in pool water as detected using polycation-sensitive ISOs soaked in 100 mM phosphate buffer, pH 7.5, with 2 mM NaCl. Each data point is derived from hue values extracted from three different photographs of the same sensor taken five minutes after initial addition of the sample to the sensor. The x-axis is plotted logarithmically (base 10).

response curve of PQ-6 in distilled water matches closely with the curve generated from PQ-6 dissolved in pool water. Of course, further testing of samples with greater pH differences and/or innate buffering capacities will need to be examined to determine the limits of this sample buffering approach.

5.4 Conclusions

PQs are important polyelectrolytes that have many applications. In this study, we have described the use of a new type of plasticizer-/polymer-free polycation-sensitive ISO as a universal technology by which various PQ species can be detected/quantified and characterized. Polycation-sensitive ISOs provide a number of benefits for PQ detection including their ability to be used for in-field analysis (using a cell phone as a detector to read the color change) and their low cost. In addition, this technology requires little or no specialized training to use.

The polycation-sensitive ISOs employed here to detect PQ species are novel in that they do not require a plasticizer or polymer and can be inkjet-printed on cellulose paper. This is quite different from polyion-sensitive polymeric membrane-based ISEs and optical sensors previously reported.[16,18,19,32–36] Using the new polycation-sensitive ISOs, PQ and DS levels are able to be detected in the ppm range with good reproducibility.

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CHAPTER 6

Polyion Detection *via* All-Solid-Contact Paper-Based Polyion-Sensitive Polymeric Membrane Electrodes

6.1 Introduction

Polyion sensing technologies have grown into a well-established field in which a wide variety of electrochemical and optical sensing methods have been developed. The bulk of research and development for these sensing technologies has largely been focused on biomedically relevant polyions (e.g., protamine,[1,2] heparin,[3,4] etc.). Although biomedical polyions are quite important, there is a large number of other types of polyionic species that have found widespread use in various industrial and cosmetic applications.[5–10] Therefore, the expansion of polyion sensing technology to the detection of these polyions in a variety of matrices such as personal care product formulations or water treatment processes can assist in developing new applications of polyion sensors as well as spark the development of new types of polyion sensing methodologies for fundamental and/or applied studies.

New configurations of polyion sensors targeted toward biomedical polyion analyses has long been an area of development. Some of these configurations include coated wire electrode

All experiments, data analysis, and figure construction for this chapter were performed by Stephen A. Ferguson. Mark E. Meyerhoff was the principal investigator for the project. The Rackham Merit Fellowship at the U-M funded the entirety of the study.

heparin sensors,[11] fully reversible all-solid-contact pulsed galvanostatic protamine sensors,[12,13] and solid contact silicon chip-based heparin sensors.[14] Sensors directed toward more industrial/cosmetic polyions (i.e., polyquaterniums (PQs)) have, to date, not undergone the same rate of targeted sensor development and application. To our knowledge, sensor configurations directed toward PQ analysis has only recently become an area of active exploration.[15] Continuing to expand advancements in new sensor configurations will allow analysts to choose the configuration for PQ analysis that best fits the desired application more readily than relying on a small, restrictive selection of conventional configurations.

All-solid-contact polyion-sensitive ISEs represents an important development for polyion analysis and characterization. More specifically, paper-based sensing devices have seen an increase in development in recent years, as paper has a wide number of beneficial properties (e.g., affordability, flexibility, biocompatibility, etc.).[16] Indeed, paper-based optical devices for PQ measurements have already been demonstrated by Ferguson et al.[15] (see Chapter 5). Hence, paper-based electrochemical devices are a logical next progression. In this chapter, the fabrication and application of the first all-solid-contact single-use polyanion-sensitive membrane electrodes are described. These all-solid-contact devices are constructed from cellulose filter paper and do not require an inner filling solution in contact with an internal Ag/AgCl reference electrode (RE) to act as an ion-to-electron transducer. Single-walled carbon nanotubes (SWCNTs) are used in this study in place of the inner filling solution and Ag/AgCl RE. This was first explored potentiometrically in the development of all-solid-contact ISEs for K^+ determination by Crespo et al.[17] The proposed sensors within this chapter are capable of detecting a variety of polyanions in a simple buffered background electrolyte. These sensors are also demonstrated to be capable of

indirectly detecting PQ species *via* potentiometric titrations in the same buffered background electrolyte solution using DS as the polyanionic titrant, with PQs being detected in the ppm range.

6.2 Experimental Section

6.2.1 Chemicals and Reagents

Whatman™ qualitative filter paper (grade 5; diameter: 240 mm; thickness: 200 μm), sodium chloride (NaCl), and DS sodium salt from Leuconostoc, were purchased from Fisher Scientific (Waltham, MA). High molecular weight poly(vinyl chloride) (PVC), bis(2-ethylhexyl) sebacate (DOS), poly(2-methacryloxyethyltrimetrylammonium) chloride (PMETAC), PQ-10, PQ-6, PQ-2, SWCNT conductive aqueous ink, heparin sodium salt from porcine intestinal mucosa, λ-carrageenan, chondroitin sulfate (CS) sodium salt from shark cartilage, and anhydrous tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate were products of Amresco, Inc. (Solon, OH). Tridodecylmethylammonium chloride (TDMAC) was a product of Polysciences (Warrington, PA). Semiconductor wafer tape was obtained from Semiconductor Equipment Corporation (Moorpark, CA).

6.2.2 Preparation of All-Solid-Contact Polyanion-Sensitive ISEs

Parent base sensors were batch fabricated by first cutting approximately 9.0 cm x 15.5 cm rectangles from a sheet of the Whatman™ qualitative filter paper. These paper rectangles were coated with one layer of carbon ink on each side using a conventional paint brush. Each coated rectangle was allowed to air dry for 10 min, followed by oven drying for 10 min at approximately 60 °C. The electrical resistance of three coated rectangles was measured using a handheld

multimeter (Hewlett Packard, Palo Alto, CA). The process of coating each side of the rectangle and air/oven drying was repeated an additional 5 times for a total of 6 coats; paper resistance was measured after each oven drying process. Once dried, individual base sensors were cut from each coated rectangle (approximately 1.0 cm x 4.5 cm).

Sensing membrane cocktails were prepared by mixing 66% PVC, 32.5% DOS, and 1.5% TDMAC (w/w) in 8 mL of anhydrous THF and allowed to completely dissolve overnight. Once dissolved, 30 µL aliquots of the membrane cocktail were drop-cast directly onto one end of one side of each individual paper-based sensor. The THF was allowed to evaporate for no less than 5 min. Additional single aliquots of 30 µL each of the membrane cocktail were drop-cast on top of the previous layers and the anhydrous THF was allowed to evaporate for no less than 10 min. A single piece of semiconductor wafer tape was placed on the side of the paper-based polyanion-sensitive ISE that did not contain the sensing membrane; the top portion of the sensor was left

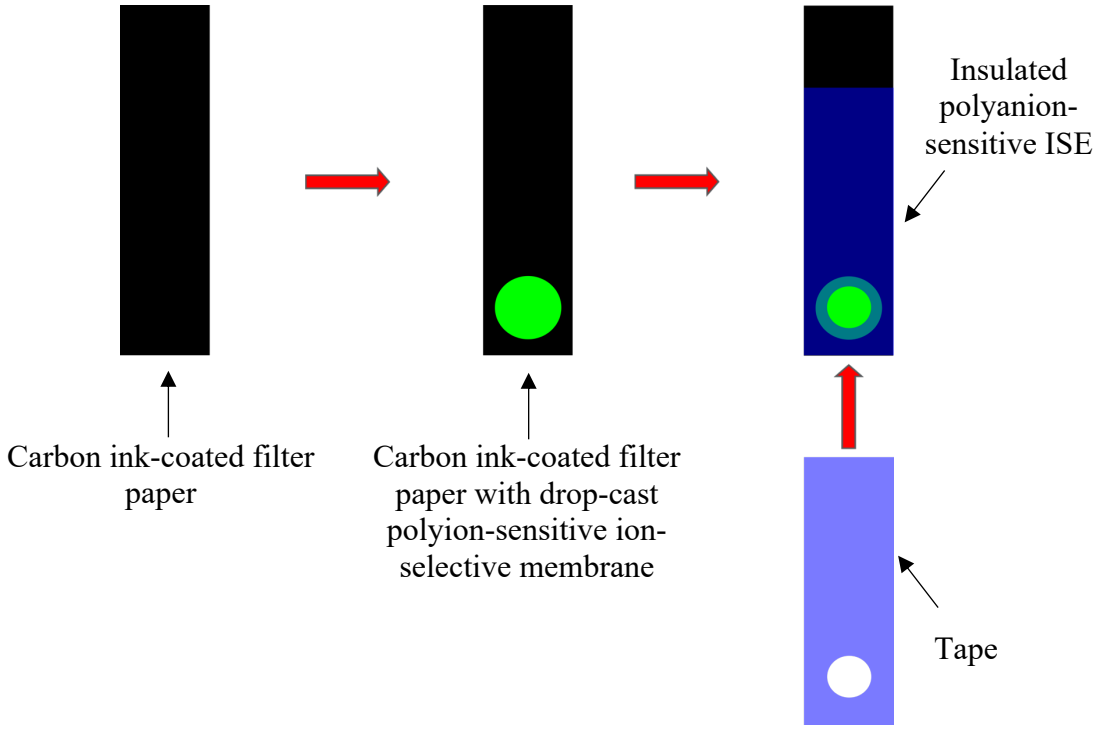

Figure 6.1. Fabrication process of paper-based all-solid-contact polyanion-sensitive ISEs.

exposed to facilitate electrical contact between the voltmeter and base sensor using a standard alligator clip. A hole of approximately 1 mm in diameter was punched into another piece of semiconductor wafer tape. This piece of tape was used to cover the remaining side of the paper-based polyanion-sensitive ISE; the hole was oriented directly over the center of the cast membrane. Figure 6.1 shows the overall sensor construction process.

6.2.3 Direct Polyanion Detection

The 10 mM phosphate buffer, pH 7.5 (with 10 mM NaCl) (10 mM PBS) used as a diluent for all experiments was prepared by diluting a 100 mM phosphate buffer, pH 7.4, containing 100 mM NaCl solution unless otherwise noted. Dose-response experiments for DS, heparin, CS, and λ-carrageenan were performed by first aliquoting a 50 mL solution of 10 mM PBS within a glass beaker. A gel-filled double junction RE was placed into the 10 mM PBS solution with three paper-based polyanion-sensitive ISEs connected to a high impedance voltmeter using an alligator clip. The inner compartment of the double junction RE contained a gel composed of 3 M KCl and the outer compartment was composed of 10% KNO_3 solution. Prior to the addition of a polyanion for a dose-response experiment, a stable baseline for each sensor was allowed to develop over 5 min when the EMF of each paper-based polyanion-sensitive ISE was measured against the double junction RE. Once stable baselines were established for each sensor, each polyanion was injected into the 10 mM PBS sample solution using a dual-syringe infusion/withdrawal pump (Cole-Parmer, Vernon Hills, IL) at a flow rate of 239 μL/min. Each ΔEMF value was calculated by averaging the last 10 pseudo-equilibrium values (sampled every 5 s) of each 1 min interval after pump initiation. The concentration of the polyanion corresponding to each ΔEMF value was

calculated based on the pump flow rate and the time at which each average EMF value was recorded.

6.2.4 Indirect Detection of PQs

Indirect detection of four different PQs was performed using potentiometric titrations. Samples containing increasing concentrations of PMETAC, PQ-10, PQ-6, and PQ-2 (see Figure 1.6 for structures) were contrived in 10 mM PBS in glass beakers at final volumes of 50 mL. The same gel-filled double junction RE used in direct detection experiments was immersed in each sample and three paper-based polyanion-sensitive ISEs connected to a high impedance voltmeter using an alligator clip were also placed within each sample. Stable baselines for each sensor were allowed to develop over 5 min when the EMF of each paper-based polyanion-sensitive ISE was measured against the double junction RE. Each sample was then titrated with DS as delivered by the same syringe pump used in direct detection experiments at a flow rate of 239 μL/min.

6.3 Results and Discussions

6.3.1 Direct Polyanion Detection

All-solid-contact polyion-sensitive ISEs have a number of benefits for both in-field analyses and point-of care diagnostic testing. In their construction, paper is a notable target as an inexpensive and robust mechanical support material. This requires the mechanical support to be electrically conductive. As cellulose paper does not exhibit inherent conductivity, it is necessary to impart electrical conductivity by the application of a conductive material (i.e., carbon ink). Figure 6.2 shows how the resistivity of the cellulose paper decreases with each successive application of the carbon ink layers. The resistivity of the cellulose paper begins to plateau after

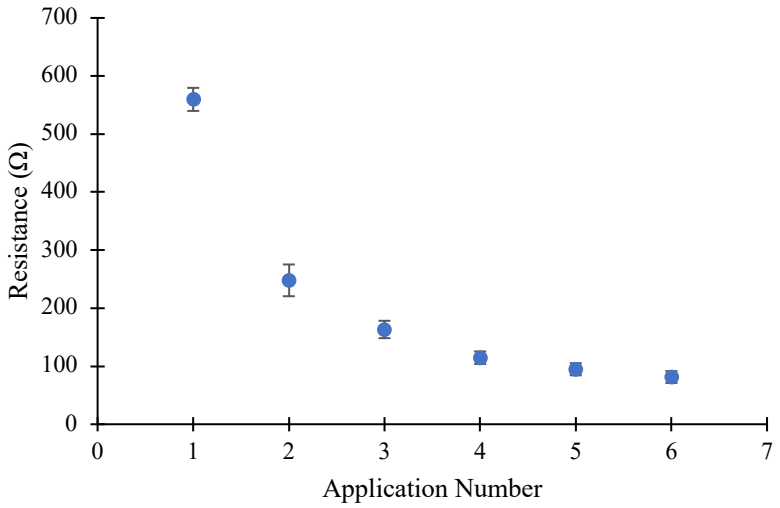

Figure 6.2. Resistivity of cellulose paper after successive applications of carbon ink. Each data point represents the average resistivity (± s.d.) of three different rectangles of cellulose paper coated in carbon ink.

the fourth application; additional coats after the fourth application did not decrease the resistivity significantly.

Once the sixth coat of the carbon ink was completely dried, the addition of the polyanion-sensitive membrane and the subsequent insulation of the sensor with tape was performed. The polyanion-sensitive membrane used for the construction of these sensors is designed for single-use polyanion detection. These sensors were used in conjunction with a gel-filled double junction RE to obtain their dose-response toward four different polyanions. Figure 6.3 shows the resulting response curves toward each polyanion. Each polyanion generates a super-Nernstian response where each data point represents the average ΔEMF per unit concentration of added polyanion (average signals ± s.d. from n = 3 sensors).

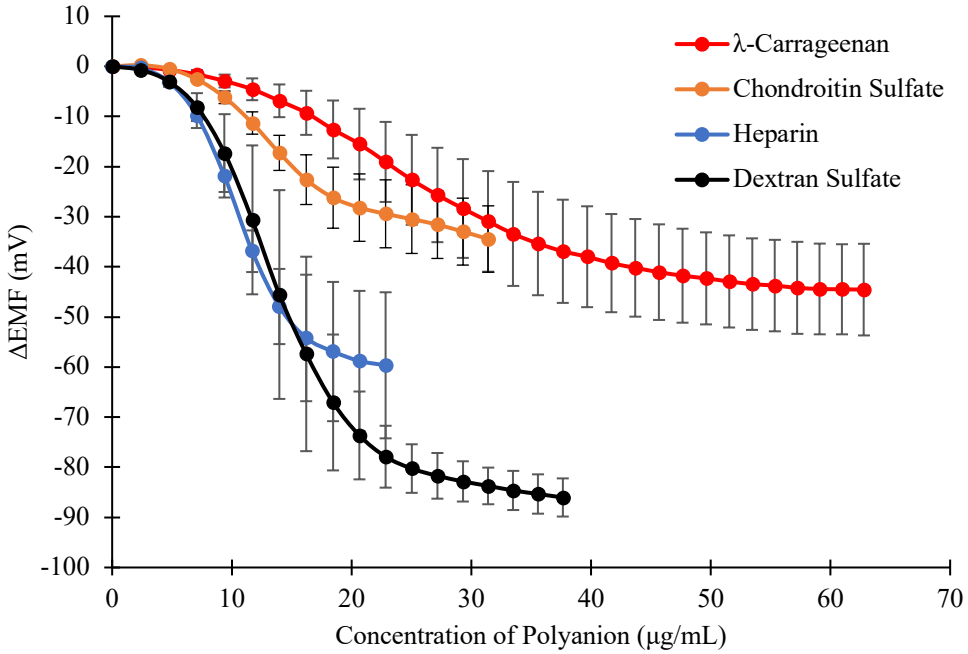

Figure 6.3. Dose-response curves of four different polyanions. Each data point represents the ΔEMF (± s.d.) per concentration of polyanion from three different polyanion sensors placed in the same solution.

6.3.2 PQ Quantification

Single-use polyanion-sensitive ISEs constructed using liquid contact macroelectrodes (Oesch Sensor Technology, Sargans, Switzerland) have already been shown to be useful in the indirect detection of a variety of cosmetic/industrial polycations (i.e., PQs).[18,19] These studies use potentiometric titrations whereby each PQ species is titrated with a polyanion and the concentration of free polyanion is monitored by a single-use polyanion-sensitive ISE. The proposed all-solid-contact polyanion-sensitive ISE should also be able to generate analogous data with similar limits of detection. Figure 6.4 shows potentiometric titrations performed by titrating various PQ species with DS using the new paper-based sensors as single-use detectors. Each generated titration curve shifts to the right of each plot as the concentration of the PQ species increases. Figure 6.5 shows the calibration curves for each PQ species generated by plotting the

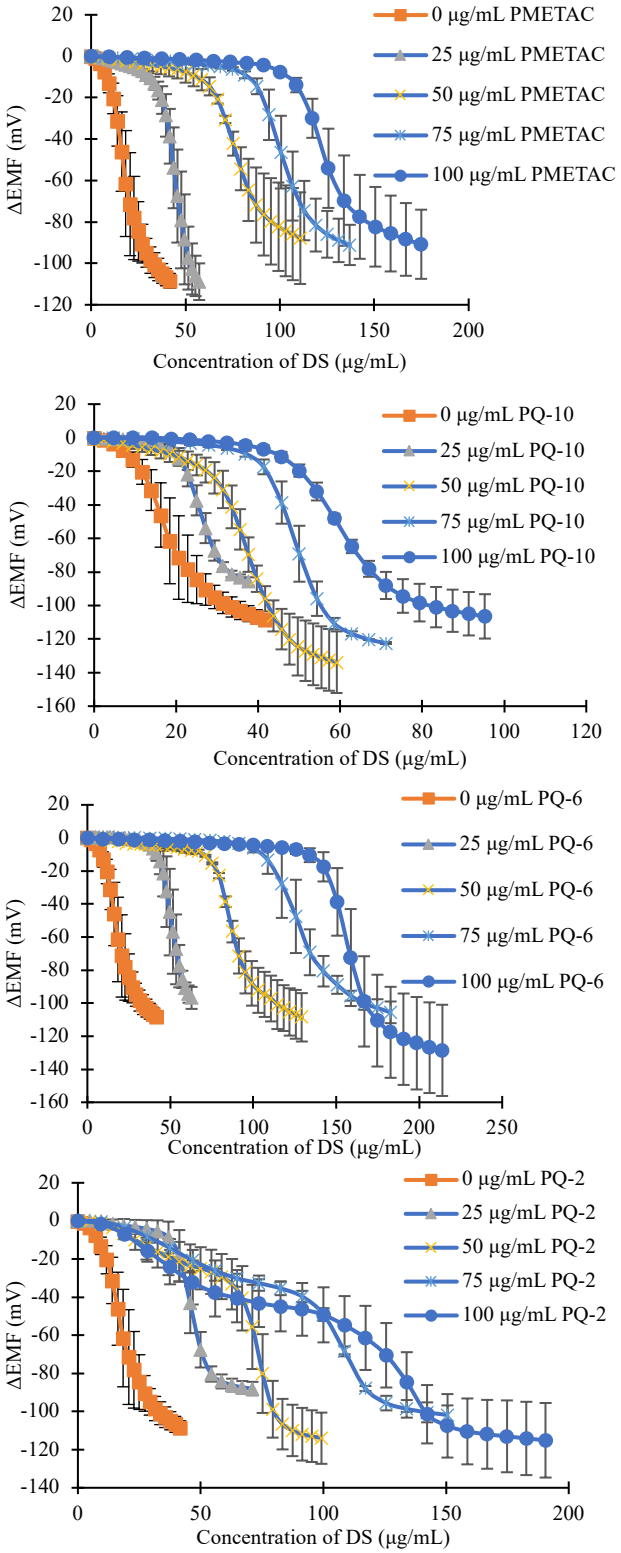

Figure 6.4. Titration curves of four PQ species titrated with DS. Each data point represents the average of three ΔEMF (± s.d.) units per unit concentration of free DS in the sample phase. Each data point represents the average ΔEMF per unit concentration of polyanion from three different polyanion sensors placed in the same solution.

first derivative of each titration curve to estimate the equivalence point. These data demonstrate that PQ quantification is possible using indirect, titrimetric methods with limits of detection (3σ) for PMETAC, PQ-10, PQ-6, and PQ-2 being 17.66, 38.08, 12.17, and 14.56 µg/mL, respectively. Further, the slope values of the calibration curves for PMETAC, PQ-10, PQ-6, and PQ-2 generated using the proposed paper-based polyion sensors using DS as the polyanionic titrant are all in excellent agreement with the slope values generated using conventional single-use macroelectrode-type polyanion-sensitive ISEs.[18] The slope values of each calibration curve generated using the proposed single-use all-solid-contact polyanion-sensitive ISEs also retain the same descending order in reference to the magnitude of the slope. This suggests that these sensors yield the same relative charge density information as can be found in earlier potentiometric

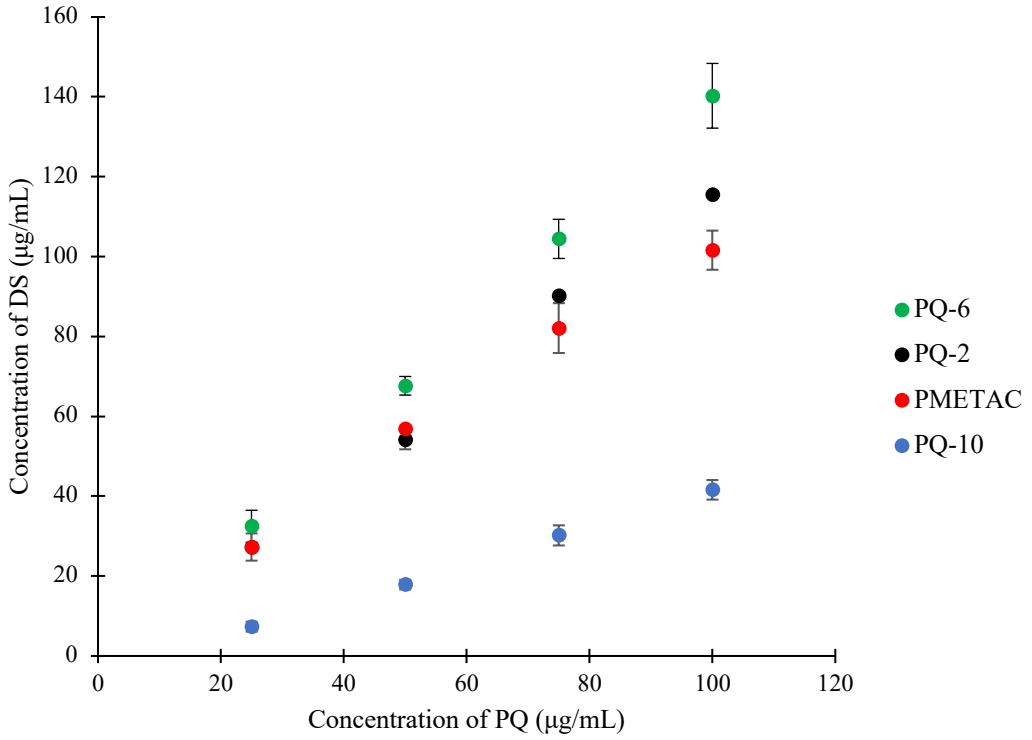

Figure 6.5. Calibration curves of four different PQs. These data are calculated based on the titration curves found in figure 6.4.

studies.[18,20] Lastly, PQ-2 titration curves yield the same shape as can be seen in earlier titration experiments, resulting from the decreased equilibrium constant between PQ-2 and DS.[18]

6.4 Conclusions

All-solid-contact paper-based polyanion-sensitive membrane electrodes have been shown to be useful in the direct detection of polyanions and the indirect detection of polycations (i.e., PQs). These sensors are completely disposable, single-use devices that can be employed as electrochemical polyanion detectors in the same fashion as their liquid contact macroelectrode counterparts.[18,19] These paper-based polyion sensors have a variety of advantages including not requiring an inner filling solution in contact with an internal Ag/AgCl RE, their ability to be batch-fabricated in large quantities, and their low cost fabrication. These new paper-based sensors have demonstrated the ability to generate analogous potentiometric data as the more conventional single-use polyanion-sensitive polymeric membrane electrodes used in earlier studies.[18] Further optimization and experimentation is required to determine the limits of these sensing technologies and how changing various parameters (e.g., number of coats of carbon ink, polyanion-sensitive membrane application number, polyanion-sensitive membrane volume per aliquot, etc.) might impact sensitivity and/or detection limits of the proposed all-solid-contact polyanion-sensitive ISEs.

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CHAPTER 7

Conclusions and Future Directions

7.1 Introduction

Conventional ISEs have long been the analytical technique of choice to measure small ionic species (e.g., K^+, Cl^-, Ca^{2+}, etc.) in aqueous solution or biological samples. Over the last several decades the use of ISEs has grown to include a staggering number of routine tests in a diverse number of fields.[1] While some polyions have vastly important roles in biological/biomedical procedures, their measurement by potentiometry using conventional ISEs has historically been avoided as it was thought the electrochemical response using such sensors under the equilibrium conditions governed by the Nernst equation (see Equation 1.1) would yield extremely small slope values owing to the high charge (z) of the polyion structures. However, it was found that if a simple lipophilic ion-exchanger was used in place of an ionophore within a polymeric membrane and the operation of the ISE was switched from operating under equilibrium conditions to operating under nonequilibrium conditions, much higher slope values could be generated.[2,3] This super-Nernstian response provides a more analytically useful electrochemical polyion response that can be used for practical analytical applications. Pioneering work in the development of these new polyion-sensitive ISEs has continued *via* a number of studies which have paved the way for the burgeoning field of electrochemical polyion sensing technologies.[2–9]

Even optical polyion sensing technologies have been developed to complement the newly established field of electrochemical polyion sensing.[10,11]

Since the first reports of both electrochemical and optical polyion sensors, development and application of these sensor technologies have been focused primarily on biomedical/biological polyion analysis. This has created a gap in the application of these devices for measurements of industrial/cosmetic polyions. Chapter 2 of this thesis partly addressed this gap by developing a methodology to first separate sodium lauryl sulfate (SLS) from polyquaternium-10 (PQ-10) in certain samples using an ion-exchange resin and then quantifying the remaining PQ-10 using polyanion-sensitive ISEs. In this study, dextran sulfate (DS) was used as a polyanionic titrant and the concentration of free DS in solution was monitored throughout each titration experiment using polyanion-sensitive ISEs. The non-adsorption of PQ-10 on the ion-exchange resin was verified by titrating one aliquot of a 25 μg/mL PQ-10 solution dissolved in 10 mM NaCl treated with the ion-exchange resin with DS as well as an identical aliquot which was left untreated for comparison. The difference between the estimated equivalence points of each titration curve was determined to be 4.3%. This suggested that the PQ-10 concentration in the sample was unaltered throughout the ion-exchange process. Chapter 2 also demonstrated that the SLS in solutions containing constant amounts of this species and increasing amounts of PQ-10 could be reduced to levels where there was negligible polyanion-sensitive ISE interference from the SLS species by using the ion-exchange resin. The remaining PQ-10 was then able to be titrated, resulting in titration curves where the estimated equivalence points were directly proportional to the concentration of PQ-10 in the original sample. Lastly, a syringe pump was also demonstrated to be capable of delivering the titrant for indirect PQ-10 titration measurements.

Chapter 3 expanded the use of polyion-sensitive ISEs from the detection of a single PQ species to the detection of a large variety of PQ species. This was accomplished by using single-use polycation-sensitive ISEs in direct PQ detection experiments and single-use polyanion-sensitive ISEs in indirect (titration) PQ detection experiments. While all tested PQs were detectable, the large variety of PQ species commercially available and the diverse nature of their chemical and physical properties presented challenges when trying to provide a universal method of direct PQ detection using polycation-sensitive ISEs. For example, if the charge density of a particular PQ is very low and the same PQ species also has a high molecular weight, the total ΔEMF generated for this species using a polycation-sensitive ISE as the detector would be too low to be considered analytical useful.[12] Therefore, indirect detection of several PQs using polyanion-sensitive ISEs as detectors in potentiometric titrations with DS serving as a titrant was the preferred analytical method.[12] DS was able to generate significantly high total ΔEMF signals and could be used to generate classical sigmoidal potentiometric titration curves, with the endpoints being directly proportional to the concentration of polycation present in the sample. Chapter 3 also demonstrated the applicability of polyion-sensitive ISEs to the indirect detection of PQ-6 in water samples obtained from a drinking water treatment plant.[12]

Although single-use polyion-sensitive ISEs are simple and sensitive devices which can be used for routine polyion detection, they cannot be readily employed to conduct semi-automated, automated, or high-throughput testing, since a new membrane/device is required for each measurement. The research described in Chapter 4 attempted to overcome this limitation by employing fully reversible polyion-sensitive ISEs (pulstrodes) in the detection of PQ species.[13] The pulstrode system employed in this study was capable of detecting both polycations and polyanions in accordance with the direction of current being applied to the sensing film.[13] As the

film was composed of both lipophilic cationic and anionic ion-exchanger species, the direction of current can be switched resulting in either a polycation sensing mode or a polyanion sensing mode.[13–15] Electrical pulses that serve to polarize the membrane were controlled by a bipotentiostat. Similar direct and indirect detection data to previous studies[12] examining a set of PQ species *via* single-use polyion-sensitive ISEs was generated using the pulstrode configuration.[13] In this work, polyanionic heparin was used as a titrant for indirect detection experiments. Further, the titration method with the reversible pulstrode sensor was shown to be capable of quantifying PQ-6 in swimming pool water using heparin as the titrant. Further, this approach was implemented in a flow-injection analysis (FIA) system.[13] The FIA system was used to directly detect polyanionic heparin in discreet sample plugs injected into a carrier stream by a six-port injection valve using the polyanion detection mode in addition to indirectly detecting/quantifying PQ-6 in discrete sample plugs by detecting free heparin in each plug that also contained varying amounts of PQ-6 (also in polyanion detection mode).[13]

Chapter 5 detailed the application of the first polymer-/plasticizer-free polycation-sensitive optical sensor to the detection of PQs. These optical devices were novel in that they do not require a polymer matrix or any accompanying plasticizer to facilitate polycation detection. Rather, the sensing ingredients (chromoionophore I and dinonylnaphthalenesulfonic acid (HDNNS)) were simply adsorbed to the surface of cellulose paper.[16] Polycations partition into the sensing layer and interact with the $DNNS^-$ species, causing the protonated chromoionophore I to release its proton to the sample phase.[16] This proton release results in a measurable color change from blue to purple.[16] These polycation-sensitive ISOs were shown to be capable of directly detecting PQs in addition to indirectly detecting DS. This chapter also demonstrated the ability of

these sensors to quantify PQ-6 in swimming pool water and provide local buffering for samples to circumvent the need for sample pretreatment (i.e., buffer addition to each sample).[16]

Finally, Chapter 6 detailed the fabrication of the first paper-based, completely disposable polyanion-sensitive ISEs. These sensors were created using cellulose filter paper as a mechanical support for polyanion-sensitive membranes deposited on the paper. This mechanical support was coated with single-walled carbon nanotubes which served as the ion-to-electron transducer.[17] The polyanion-sensitive membrane was then cast on top of the carbon nanotube layer and finally the remainder of the surface was insulated with a layer of tape. These devices were used as working electrodes in two-electrode potentiometry experiments for polyanion detection. This chapter demonstrated that these new single-use sensors could directly and indirectly (titration) detect polyanions and polycations, respectively. These sensors were also shown to be capable of generating titrimetric data that was in agreement with earlier single-use macroelectrode polyion-sensitive ISE experiments.[12]

7.2 Future Directions

7.2.1 Interferents in Personal Care Product Formulations

One notable application of polyion-sensitive ISEs is the analysis of PQs formulated within personal care product matrices. As discussed throughout this dissertation, PQs are well known to be useful in a variety of consumer goods/personal care products (e.g., shampoos/conditioners) (see Chapter 1).[18] The formulations into which PQs are added often contain a large variety of additional ingredients that help deliver tangible and perceived customer benefits (opacifiers, surfactants, etc.).[19] As these ingredients have a range of chemical and physical properties, it is reasonable to expect that a single ingredient, or a combination of ingredients, may interfere with the analytical

signal measured by polyion-sensitive ISEs. The interference might also override the signal that would otherwise be solely generated by the analyte polyion. For instance, nonionic surfactants are common additives in surfactant-based personal care product formulations,[20,21] and have been shown to interfere with EMF signals of polymeric membrane-based ISEs.[22–24] Ionic surfactants such as sodium lauryl sulfate (SLS) (see Chapter 2) can also present challenges when measuring various analytes using polyanion-sensitive ISEs.[25] Proteins can even affect the stability of potentials measured by polymeric membrane-based ISEs.[26] Given the diversity of the types of interferents that may be present in a given personal care product sample, it is imperative to assess the compatibility of polyion-sensitive ISEs with various components within these formulations so that their applicability to the analysis of certain industrial formulations can be verified.

7.2.2 Selectivity of Polyion-Sensitive ISEs

Selectivity is of utmost importance when considering conventional ISEs targeted toward the detection of small ions (e.g., K^+, Cl^-, Ca^{2+}, etc.). This particular characteristic is usually provided by an ionophore capable of reversibly and selectively binding a particular ion.[1,27] A classic example is valinomycin for K^+ determination. The ability of conventional ISEs to selectively determine one ion over various interfering ions in sample matrices as complex as whole human blood has allowed the use of these chemical sensors to flourish in a host of commercial whole blood analysis instruments. Though polyion-sensitive ISEs are similar to conventional ISEs in their construction, membrane formulation, and operation, polyion-sensitive ISEs do not exhibit significant selectivity between different polyions. While some polyion-sensitive ISEs have marked selectively over small ions such as Cl^-,[2] there is little selectivity between different polyions of the same charge. Some experiments have been performed in altering the background electrolyte

concentrations in which polyion detection occurs to improve EMF selectivity of some polyions over others.[6] However, this requires complex sample testing procedures involving a variety of solutions cotaining different background electrolyte concentrations. In addition to the enhanced selectivity resulting from the alteration of the background electrolyte concentration, these sensors can also exhibit selectivity for higher molecular polyion fragments over lower molecular weight fragments of the same polymer (e.g., high vs. low molecular weight heparin).[2] This occurs until a critical molecular weight (low end) is reached where little to no potentiometric response is observed.[2,5]

While smaller molecular weight fragments yield reduced EMF responses in polyion-sensitive ISEs when compared their higher molecular weight counterparts,[2] there is currently no feasible method that can differentiate two different polyionic species of like charge and similar molecular weights. In addition, the manipulation of background electrolyte concentrations requires labor intensive (and time consuming) sample preparation procedures. Molecularly imprinted polymer (MIP)-based potentiometric sensors can address selectivity concerns in a reversible and reproducible way.[28] Indeed, potentiometric MIP-based sensors for atrazine,[29] heparin,[30] and trypsin/yeast[31] have already been shown to be selective for their respective target analytes. Further research and development in expanding MIP-based potentiometric sensing devices may hold promise in developing truly selective polyion-sensitive ISEs, beyond the heparin sensor already reported.[30]

7.2.3 PQ Degradation Studies

Since PQ species are common additives in consumer goods/personal care product formulations it is important to develop quality control (QC) procedures to quantitate and detect

these species in such sample matrices. Early potentiometric studies have been performed to demonstrate that PQs can be quantified using single-use and fully reversible polyion-sensitive ISEs (see Chapter 2-4).[12,13,25] These studies provided the capability to determine the amount of a particular PQ species within a formulation, thus providing an analytical means by which to conduct routine QC tests for a manufactured product. Another benefit that polyion-sensitive ISEs (both single-use and fully reversible) can potentially provide is the determination of PQ degradation over time within formulations.

Earlier studies have demonstrated the ability of single-use polyion-sensitive ISEs to differentiate between low molecular weight vs. high molecular weight fragments of heparin.[2] Within these studies it was determined that there is a critical chain length of polyanionic heparin required to generate a negative potential change by observing the direct potentiometric response of heparin fragments having varying lengths. In this study, heparin fragments were obtained by hydrolyzing heparin with nitrous acid.[2] This can also be applied to studies examining potential PQ degradation over time within different formulations. This could assist in the determination of an optimal shelf life of a particular product. For example, some hair relaxing formulations incorporate PQ-6 into their formulations as a preferred conditioning agent. Hair relaxing formulations typically have high pH values; these pH values can range from 12 to 14.[32] PQ-6 is relatively stable between pH values 3 and 12.[33] Formulating PQ-6 into hair relaxing formulations with pH values exceeding this range might result in polymer degradation processes.[33] This potential degradation of PQ-6 at high pH values, and possibly other PQs formulated in initial exploratory efforts throughout product development stages, can potentially be monitored using single-use polycation sensitive ISEs in much the same way that heparin fragments of varying size were monitored in earlier experiments.[2] This monitoring can also potentially be expanded to the pulstrode system.

7.2.4 EMF Stability and Reproducibility in All-Solid-Contact Polyanion-Sensitive ISEs

All-solid-contact polyion-sensitive ISEs are an important development in polyion sensing technology. The newly proposed paper-based, single-use polyanion-sensitive ISEs developed in Chapter 6 have demonstrated the ability to generate analogous potentiometric titration data for PQs to the single-use, macroelectrode polyanion-sensitive ISEs employed in Chapter 3 studies.[12] However, these sensors are not able to generate reproducible absolute EMF values from sensor-to-sensor. This presents a significant challenge in developing paper-based, calibration-free polyion sensing devices. Calibration-free ISEs targeted toward small ion detection have been the focus of considerable research and development as they hold promise in circumventing the need for frequent calibration procedures in routine testing environments (e.g., clinical laboratories) or for use in home testing.[34]

There have been some studies in examining EMF stability and reproducibility of solid-state ISEs for NH_4^+ and Na^+ determination.[35] In these studies, various Ag^+-ligand complex/free ligand pairs were incorporated into NH_4^+ and Na^+ sensing films to determine how absolute EMF reproducibility and EMF drift would be affected.[35] It was determined that the free thioether derivatized calix[4]arene (i.e., not complexed with Ag^+) demonstrated higher selectivity toward Ag^+ over other ions (NH_4^+ or Na^+) in addition to possessing the highest lipophilicity between itself and 1,3-(bis-phenylphosphine) propane and was therefore the preferred ligand for Ag^+.[35] This ligand also resulted in reduced EMF drift and improved absolute EMF reproducibility between sensors.[35] Another study applied this same principle for the detection of polyionic heparin and protamine using solid-state polyion sensors.[36] These polyion sensors were based on silver epoxy electrodes printed on glass wafers and cast with various polyion sensing films containing the same

Ag^+-thioether derivatized calix[4]arene complex/free thioether derivatized calix[4]arene (among other sensing ingredients) and showed improved EMF stability and absolute EMF reproducibility.[36] Applying these approaches to the proposed all-solid-contact polyanion-sensitive ISEs described in Chapter 6 could potentially reduce EMF drift found in these sensing devices (see Figure 7.1 as example) as well as provide a means by which calibration-free all-solid-contact polyanion-sensitive ISEs can be developed.

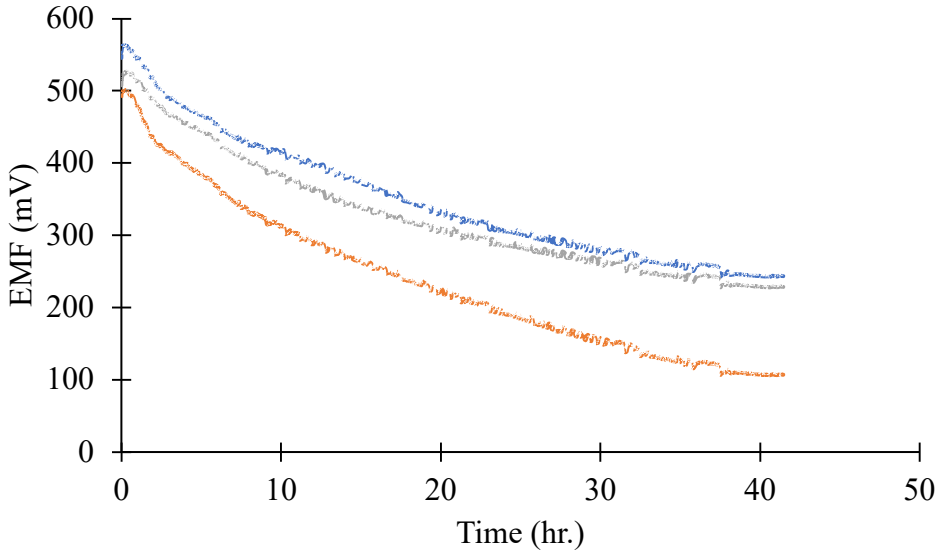

Figure 7.1. The drift in EMF and absolute EMF irreproducibility exhibited by three different all-solid-contact polyanion-sensitive ISEs placed in 10 mM phosphate buffer, pH 7.5 (with 10 mM NaCl).

7.3 References

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